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THE CARDIAC OUTPUT IN MAN

CHANGES IN ALVEOLAR OXYGEN AND CARBON DIOXIDE TENSIONS DURING REBREATHING AND THE BEARING OF THESE UPON THE TRIPLE EXTRAPOLATION METHOD OF ESTIMATING CARDIAC OUTPUT

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Since the enunciation of Fick's principle in 1870 many methods, dependent upon a knowledge of the oxygen and carbon dioxide contents of mixed venous blood, have been proposed for the determination of the cardiac output in man. Many of the procedures, however, rely upon the equilibration of the gas in the lungs with the incoming blood as regards oxygen, carbon dioxide, or both gases. The difficulty of such procedures has been pointed out by Richards and Strauss (1930).

The "triple extrapolation" method of Redfield, Bock and Meakins (1922) represented a simplification of technique, as the tensions of the gases in the mixed venous blood were inferred from analyses of mixtures having quite different gas tensions. It was assumed that, during the period of holding the breath, the variations of alveolar carbon dioxide and oxygen tensions were directly proportional to each other, and hence that the initial and final alveolar tensions for each experiment, plotted on a carbon dioxide-oxygen tension graph, defined a straight line passing through the point corresponding to air in equilibrium with the mixed venous blood. The point of intersection of two such lines was therefore believed to represent the carbon dioxide and oxygen tensions of mixed venous blood.

Grollman (1930) in using the method recognized that many of the experiments appeared to be aberrant. Finding that only occasionally did the lines representing three experiments in which the breath was held meet in a single point, he customarily performed six such experiments. For the final calculation he used only the three or four which defined lines having a common point of intersection. With this modification he secured

essential agreement of the results with values of cardiac output obtained with the use of acetylene.

Although the validity of the linear extrapolation employed in the triple extrapolation method has apparently not been questioned, it seemed worth while to examine the basis on which it rests.

THEORETICAL. One approach to the problem of determining the progressive changes in the oxygen and carbon dioxide tensions in the alveolar air during the period of breath-holding lies in calculations made from the available data regarding a normal subject. For this purpose the following assumptions were made: *a*, the venous blood is constant in its content of oxygen and carbon dioxide during the period considered; *b*, the blood leaving the lungs is in equilibrium with the alveolar air at the moment; *c*, the rate of blood flow is constant and equal to 70 cc. per second; *d*, the effective alveolar volume for both oxygen and carbon dioxide when reduced to standard conditions is 3000 cc., and *e*, the oxygen capacity of the blood is 20 volumes per cent.

In order to make as direct a comparison as possible with the results secured by the triple extrapolation method, the calculations were based upon an experiment taken from the most recent investigation in which this method was employed (Grollman, 1930 and 1932, p. 33). The graph of this experiment has been reproduced in figure 1.

The alveolar air tensions which Grollman found in the initial sample, *A*, were 36.2 mm. of oxygen and 28.1 mm. of carbon dioxide. When in addition to the assumptions given above, the tensions which he found for venous blood, namely, 38.9 mm. for oxygen and 50.3 mm. for carbon dioxide, were taken as correct, it could be calculated by means of the nomogram of Henderson, et al. (1924) that 1.0 cc. of oxygen would be absorbed from the alveolar air in the first second and 8.0 cc. of carbon dioxide would be added to it. From these values the new tensions of the alveolar air (point *A'*) were determined, and the process was continued for the next and subsequent seconds. The results, plotted for the three original samples of Grollman's experiment, are shown in the figure. It will be seen that the successive values of gas tensions *do not lie on straight lines* but on curves which, except for curve 3, do not pass near the points representing the second sample of each experiment (*D*, *E* and *F*).

Changes of 100 per cent or more in the assumed blood flow and alveolar volume, although they changed the spacing of the points computed for equal time intervals, were found to give no appreciable change in the shape of the calculated curves. A change in the assumed oxygen capacity of the blood was of course found to alter the shape of the curves, but even when the assumed difference was as great as 5 volumes per cent the alteration in shape was not significant for the purposes of this investigation.

By trial of various assumed values of oxygen and carbon dioxide ten-

sions of mixed venous blood it was found that curves constructed with the venous tensions represented by point V of figure 1 pass reasonably close to the tensions found in each second sample (D, E and F). From the nomogram of Henderson et al. (1924) point T found by linear extrapolation represents an oxygen saturation of 70 per cent, and point V an oxygen

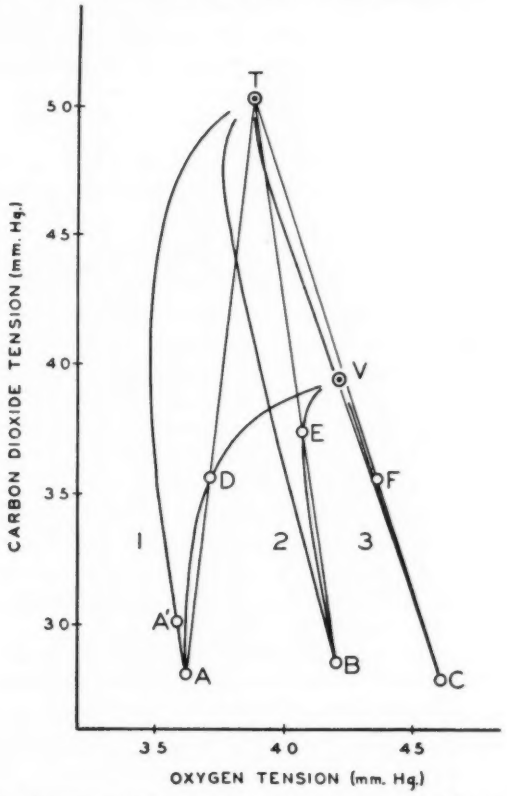

Fig. 1. A triple extrapolation experiment of Grollman (1930) by the method of Redfield, Bock and Meakins (1922).

The curved lines indicate the calculated changes of tension of oxygen and carbon dioxide in the lungs when those in venous blood are assumed to be represented by the points T and V.

saturation of 79.6 per cent. The difference in the resulting values of cardiac output would represent an error well beyond that usually assumed to be inherent in the triple extrapolation method.

Computations of the type described above have been made for a number of other values of venous tensions and for many initial gas tensions. The

variation in both the direction and magnitude of the curvature obtained, as the initial gas tensions employed in the calculations were altered, may be seen from an examination of the solid curves of figure 2. Only in exceptional cases have the curves been found to approach a straight line. That the deviations from straight lines were not due to the nomogram employed is shown by the fact that similar results were obtained when the nomogram of Dill et al. (1928, p. 207) was used. We conclude, therefore, that under the conditions of these experiments changes in the oxygen and carbon

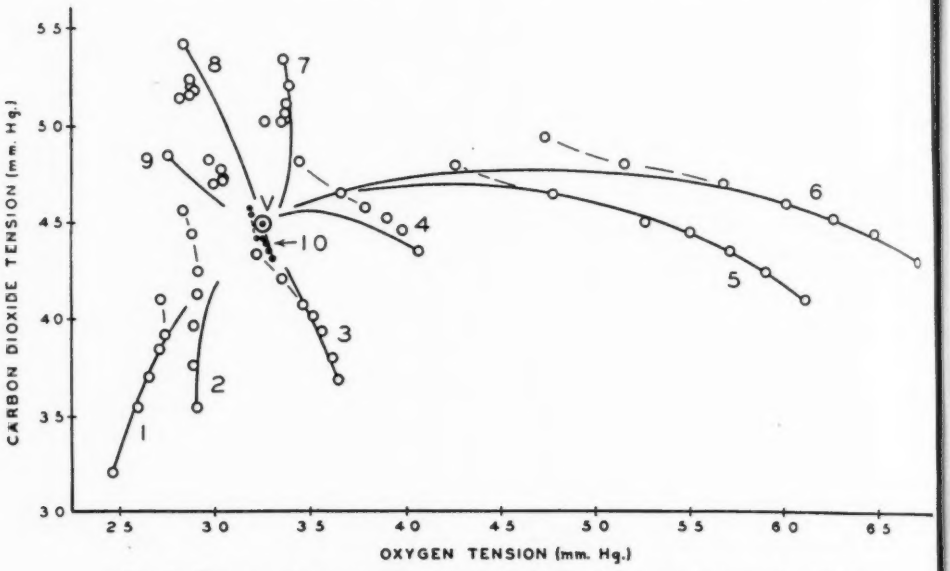

Fig. 2. Tensions of oxygen and carbon dioxide in the lungs during rebreathing experiments on the same subject on different days.

The solid curves were computed using the tensions of alveolar air found in the first sample of each experiment and assuming that the tensions of mixed venous blood were represented by the point *V*. The subject was basal in experiments 3, 7, 9 and 10; semi-basal in 1, 4, 5, 6 and 8; and non-basal in experiment 2.

dioxide tensions of the lung air should not be expected to be directly proportional to each other.

EXPERIMENTAL. Because of the impossibility of securing enough alveolar samples from a single breath, the subject was allowed to rebreathe various gas mixtures from a rubber bag and samples of successive expirations were taken after complete mixing had been assured.

A. Ten experiments on a single subject, performed on different days:

Apparatus and procedure. The apparatus was similar to that employed by Richards and Strauss (1930). Two valves made possible the connection of the subject with room air, with a small spirometer, or with a rubber bag. Seven 50 cc. sampling tubes were used. To collect the sample in a very short time these were provided with stopcocks of 4 mm. bore and connected to the wide tube adjacent to the mouth piece by 4 mm. tubing. Dilution of the sample was avoided by filling the connecting tube with mercury. A stopwatch was used to time the collection of two samples, from which the times of the remainder could be interpolated.

The gas mixture in the rubber rebreathing bag was varied so that equilibrium was approached from various combinations of initially high or low oxygen and carbon dioxide tensions.[1] After expiring maximally (zero time) the subject rebreathed from the bag, at the rate of one cycle in two seconds as governed by a metronome, and seven samples were collected from the last portions of the sixth and succeeding expirations.

The carbon dioxide tensions found in these experiments have been plotted as a function of the corresponding oxygen tensions in figure 2. Although the gas tensions of the mixed venous blood of the subject must have varied somewhat from day to day, for purposes of comparison the point *V* of the figure was chosen as representing the average point of convergence of the curves. Using this point, hypothetical curves from the first sample of each experiment have been computed by the method described and plotted in figure 2 as solid lines.

Recirculation, for which no allowance is made in the theoretical calculations, may be seen to cause deflections in the later portions of many of the experimental curves. The early portions, however, in eight out of ten of the experiments (all except 8 and 9) are essentially of the same shape and direction as those computed. That this curvature is not primarily due to inadequate mixing, to venous blood made abnormal by the temporarily accelerated circulation, or to unexpectedly early recirculation is indicated by the fact that some of the curvatures are in a direction opposite to that which each of these factors would cause. That the increase in blood flow due to rebreathing cannot alter the shape of the curves was shown on page 496.

It appears, therefore, that the curves found are not due to abnormalities resulting from the respiratory procedure or from recirculation, and that they agree closely with those anticipated on theoretical grounds.

Because Richards and Strauss (1930) chose their rebreathing mixtures so as to give an initial alveolar air close to that of final equilibrium, the curvature in their results is not so obvious as in ours, and is more subject

[1] When very low oxygen tensions were desired, one to three initial breaths were taken from the small spirometer filled with nitrogen, the elapsed time being measured from the first of these.

to distortion by analytical errors. Their results are, however, entirely compatible with those here presented.

B. Pairs of experiments on basal subjects. In order to demonstrate the differences between the tensions of oxygen and carbon dioxide in the mixed venous blood, as well as in the values of cardiac output, as estimated by linear extrapolation and by extrapolation of the experimentally determined curves, eleven pairs of rebreathing experiments, separated only by short intervals of time, were performed on ten basal subjects.

Apparatus and procedure. Immediately before the rebreathing experiments the basal cardiac output was determined in duplicate by the ethyl iodide method of Starr and Gamble (1928) using the katharometer as described by Donal, Gamble and Shaw (1934). Simultaneous determinations were made in duplicate of the oxygen consumption, carbon dioxide elimination, and the oxygen and carbon dioxide tensions in automatically collected samples of alveolar air.

The apparatus for the rebreathing experiments was identical with that described under *A*, except that a kymograph was used to record the time of collection of each sample. The rebreathings on each subject were carried out in such a range of gas tensions that the resulting curves converged at an angle of at least 45°. Convergence of the curves at a smaller angle would have greatly increased the differences between the results obtained by linear extrapolation and by extrapolation of the experimentally determined curves. Five to seven samples were collected during each rebreathing, from the last portions of the sixth and each subsequent expiration to the bag.

The results of two experiments which were representative are shown in figures 3 and 4. Only those samples were considered valid in any one subject which were collected before the earliest time at which recirculation was indicated by an inflection of either curve. Straight lines, as in the figures, were drawn through the pairs of points corresponding to the first and last valid samples as thus defined. These lines were projected to meet at the point *T*, representing the values of carbon dioxide and oxygen tension in the mixed venous blood as determined by the triple extrapolation method.

Employing the method of computation already outlined, the points *V* of figures 3 and 4 were determined by trial so that the curves, showing the courses of the alveolar gas tensions from each of the initial samples, would pass through the points representing the last valid samples.

From these two extrapolated values of the gas tensions in the venous blood, cardiac outputs were calculated (table 1). For this purpose the average of the two measurements of metabolism and of alveolar air tension which were made at the time of the ethyl iodide determination of the cardiac output, and the nomogram of Henderson et al. (1924), were used. The subject's blood was assumed to have an oxygen capacity of 20 volumes per cent and to be 96 per cent saturated when leaving the lungs. For graphic comparison, the cardiac output as measured by the ethyl iodide

method has been used to calculate the gas tensions in the venous blood which have been plotted in figures 3 and 4 as points *K*.

DISCUSSION OF RESULTS. Of the twenty-two rebreathings in this series, twenty-one showed curvature in close agreement with that predicted by the computed curves used to determine the point *V*. The results of the remaining rebreathing were divergent, the points lying on a straight line. The

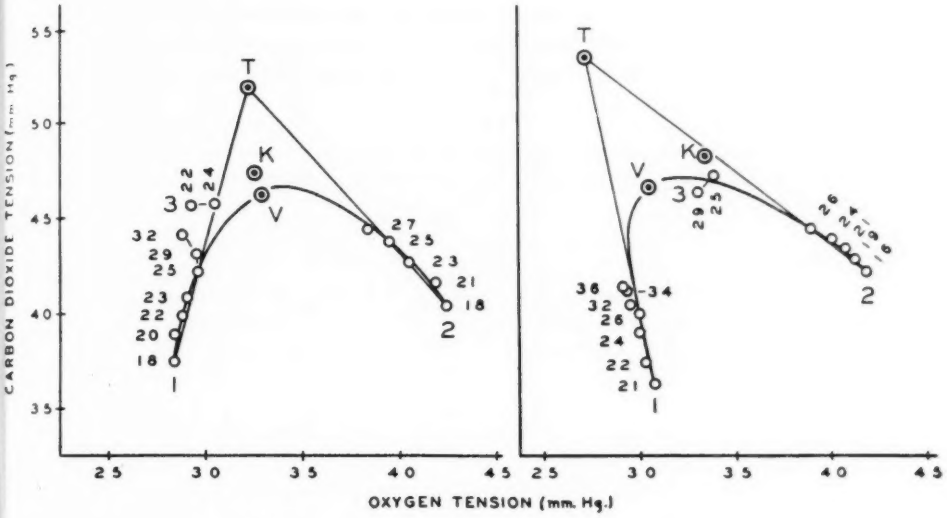

Fig. 3 Fig. 4

Figs. 3 and 4. Representative experiments on basal subjects. The tensions of oxygen and carbon dioxide in the lungs during successive rebreathing experiments on the same day.

The point *T* was determined by linear extrapolation. Using the first sample of each experiment, the point *V* was determined by trial so that the computed curves passed through the points representing the last samples collected before recirculation became evident. *K* represents the tensions in venous blood calculated from the cardiac output measured by the ethyl iodide method. The time of sample collection in seconds is shown adjacent to each point. Cardiac output data for these experiments are given in lines 1 and 3 of table 1.

validity of the computations and assumptions on which the curved extrapolations were based were supported by the close agreement of the cardiac outputs which these gave with those determined by the ethyl iodide method (table 1, columns 1, 2 and 3).

When the linear extrapolation was used the agreement with the ethyl iodide results was not so complete. In the case of the cardiac outputs based upon oxygen tensions the differences were not large. This was to

be expected from the fact that one of the curves of each pair showed relatively little change in oxygen tension (curves 1, figs. 3 and 4). Moreover, the equal division of these oxygen changes in the various experiments between small increases and small decreases improved the agreement of the averaged results.

In the case of carbon dioxide, the mixtures chosen gave (as is frequent in "triple extrapolation" experiments) tensions which were increasing rapidly in both rebreathings, and curves which were concave toward each other. Linear extrapolation therefore indicated a venous carbon dioxide in each experiment higher than that shown by the calculated curves, with the

TABLE 1

Comparison of the values of cardiac output obtained from rebreathing experiments with the estimations by the ethyl iodide method

(Liters per minute)

AVERAGE OF DUPLICATE ETHYL IODIDE ESTIMATIONS	EXTRAPOLATION OF EXPERIMENTAL CURVES		TRIPLE EXTRAPOLATION METHOD	
	From oxygen data	From carbon dioxide data	From oxygen data	From carbon dioxide data
3.3	3.4	3.6	3.1	2.4
2.9	3.7	3.6	2.9	2.3
3.6	3.2	3.9	2.6	2.4
4.0	4.5	4.5	4.2	2.9
3.3	3.9	3.2	3.0	2.0
2.9	3.5	2.8	2.9	1.9
3.5	3.4	3.7	2.6	1.9
4.8	7.2	4.1	5.9	3.2
2.6	4.0	2.9	3.4	2.3
4.0	4.4	3.7	3.4	2.9
4.9	4.4	4.4	3.3	2.4
Average 3.7	4.1	3.7	3.4	2.4

result that the calculated cardiac outputs which they indicate (column 5) are lower in each instance than those found with ethyl iodide (column 1). Statistical analysis shows that there is a probability greater than 99 per cent that the difference between the two average values is real.

Additional evidence that the points indicated by the curved extrapolation are the more correct was secured in five experiments in which a third rebreathing was performed (curves 3 of figs. 3 and 4). In each of these the carbon dioxide in the last sample was markedly below that indicated by linear extrapolation and had either decreased from the previous sample (4 experiments) or was identical with it (fig. 3). In each of the five, moreover, the tensions approached values close to those indicated by the curved extrapolation.

As their experiments were in approximately the same range of gas tensions as those given above, this effect was probably responsible for the finding of Redfield, Bock and Meakins that the "true" venous carbon dioxide tension which their method indicated agreed with the oxygenated venous carbon dioxide tension which they obtained by the method of Henderson and Prince (1917).

We would therefore conclude that accurate results are to be expected with the triple extrapolation method of Redfield, Bock and Meakins only when the initial tensions of the alveolar air are nearly in equilibrium with the mixed venous blood, or under the infrequent circumstances in which the carbon dioxide and oxygen are changing at nearly proportional rates (e.g., curve 3 of fig. 1). Adjustment of the rebreathing mixture to attain these ends would require an advance knowledge of the approximate values of the venous tensions and of the volume and composition of the residual air and is therefore difficult of attainment.

Time of recirculation. As the estimates of the duration of the period before recirculation occurs are varied, it is of interest to determine its length from work of the type here reported. In the eleven experiments here described the inflections in the carbon dioxide-oxygen curves due to blood with abnormally low oxygen content were evident after an average time, from the start of the rebreathing, of 24.5 seconds, with a standard deviation of 2.4 seconds.[2] Although the temptation is strong to compare this value with others previously reported for "recirculation" or "total circulation time," it must be remembered that the solvent action of the tissues will vary with each substance used, being high for gases like carbon dioxide and acetylene, and low for the dyes which have been employed. As Starr and Collins (1933) have pointed out, the extent of recirculation which will constitute a detectable amount will consequently vary. It is to be anticipated, moreover, that the time at which recirculation occurs will be different for each type of respiratory gymnastic employed. Application of the recirculation time found above should therefore be limited to experiments of the type here given.

[2] For carbon dioxide the inflections were less evident and appeared to occur somewhat later. In three experiments they were observed at an average time of 26.2 seconds, while the corresponding value as measured by oxygen in the same subjects was 23.7 seconds. In the remaining eight experiments no inflection indicating recirculation of carbon dioxide-rich blood was noted, although the last sample was collected at an average time of 25.6 seconds and, with one exception, was taken after the time at which recirculation of blood low in oxygen was evident in the same subject. This difference in the results secured with the two gases is probably due to the greater solvent or "reservoir" action of the tissues for carbon dioxide, an effect predicted by Douglas and Haldane (1922, p. 73).

SUMMARY

1. The simultaneous values of oxygen tension and of carbon dioxide tension in the lungs were computed for hypothetical experiments of the type used in the triple extrapolation method of Redfield, Bock and Meakins (1922) for estimating the cardiac output in man. When each computed value of carbon dioxide tension is plotted as a function of the computed oxygen tension, the points were found to lie on curves and not on straight lines as is assumed in the triple extrapolation method.

2. The plotting of analyses of successive samples taken during continuous rebreathing gave curves with the same characteristics as those calculated from theoretical considerations.

3. Pairs of such curves were constructed from experiments on 10 subjects. When these were extrapolated by the linear procedure of the triple extrapolation method the venous tensions found were different from those indicated by the convergence of the curves. Because of the tensions in the rebreathed mixtures chosen for these experiments these differences were greater for carbon dioxide than for oxygen.

4. Cardiac outputs of these subjects determined at the same time by the ethyl iodide method agreed more closely with a curved than with a linear extrapolation.

5. It is concluded from these results that when gases are rebreathed or held in the lungs, variations in carbon dioxide and in oxygen tensions cannot be assumed to be directly proportional to each other. In consequence the triple extrapolation method for the estimation of blood flow which Redfield, Bock, and Meakins have based on such an assumption cannot be expected to give reliable results.

6. In 11 rebreathing experiments, return to the lungs of blood abnormally low in oxygen content was evident at an average time of 24.5 seconds after the beginning of rebreathing. Recirculation of blood high in carbon dioxide was less readily detectable and was not evident until a later time.

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THE NERVE PATHWAYS INVOLVED IN THE PALATINE AND PHARYNGEAL RESPIRATORY REFLEXES OF THE CAT

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In a recent preliminary report Teitelbaum and Ries (1934) announced that respiration can be markedly accelerated in the cat by stimulating the pharyngeal mucosa mechanically. Since that time is has been ascertained that the soft palate gives a similar response. In addition to the accelerator response described originally one can also obtain an inhibitory respiratory response on stimulating the pharyngopalatine mucosa of the cat mechanically.

MATERIAL AND METHOD. The cats were anesthetized with ether only long enough to permit the intravenous injection of 275 mgm. per kilo of barbital—Na. While moderately deep ether anesthesia sometimes abolished the pharyngopalatine respiratory reflexes, barbital—Na anesthesia not only had no harmful effects, but even tended to exaggerate the reflexes.

Respiration was recorded by a tambour connected to a tracheal cannula. The pharyngopalatine mucosa was stimulated by means of a cotton swab inserted through the mouth, or by tapping with the finger on the inframandibular region of the neck, just cephalad to the hyoid bone.

In the operative procedure, the vagus and glossopharyngeal nerves were best approached by a suboccipital incision, while the sphenopalatine ganglia, and the Vidian and maxillary nerves were exposed by enucleating both eyes.

RESULTS. Of the 42 cats studied, 33 responded normally to mechanical stimulation of the pharyngopalatine mucosa with respiratory acceleration alone, 8 responded with acceleration and inhibition, and 1 responded with inhibition alone.

[1] Hitchcock Fellow in Gross Anatomy 1932–1933. Weaver Fellow in Gross Anatomy, 1935–1936.

[2] The authors wish to express their appreciation to Prof. O. G. Harne and Miss E Painter of the Department of Physiology for making available facilities essential for the completion of the experiments reported, and to Dr. M. Gregersen of the Department of Physiology and Dr. E. Uhlenhuth of the Department of Anatomy for reading the manuscript and making a number of helpful suggestions.

The effect of the application of cocaine to the pharyngopalatine mucosa. A 2 per cent solution of cocaine was applied to the pharyngopalatine mucosa of several cats after control tracings had been taken. Two minutes after the application of cocaine stimulation of the pharyngopalatine mucosa produced complete inhibition of respiration in place of the original acceleration. Four minutes after the application of cocaine, stimulation produced neither acceleration nor inhibition of respiration. These results indicate that the nerve endings mediating the inhibitory reflex from the pharyngopalatine mucosa are more resistant to the anesthetic effects of cocaine than are the nerve endings mediating respiratory acceleration.

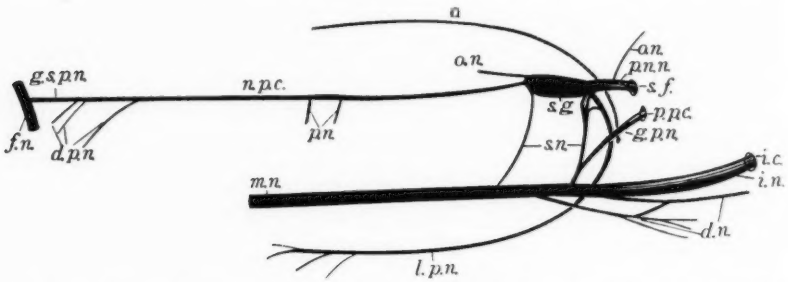

Fig. 1. Cat. Dissection of the sphenopalatine ganglion and its branches, carried out with the aid of a binocular dissecting microscope. The ganglion and the nerve in the pterygoid canal (Vidian nerve) lie parallel to the maxillary nerve on the floor of the orbit. The maxillary nerve lies lateral to the ganglion, and on the same plane.

Abbreviations. *d.n.*—dental nerves. *d.p.n.*—deep petrosal nerves. *f.n.*—facial nerve. *g.p.n.*—greater palatine nerve. *g.s.p.n.*—greater superficial petrosal nerve. *i.c.*—infraorbital canal. *i.n.*—infraorbital nerve. *l.p.n.*—lesser palatine nerve. *m.n.*—maxillary nerve. *n.p.c.*—nerve in the pterygoid canal (Vidian nerve). *o.*—orbital wall, medial. *o.n.*—orbital nerves. *p.n.*—palatine nerves (branches of *n.p.c.* which pierce palatine bone). *p.n.n.*—posterior nasal nerve. *p.p.c.*—posterior palatine canal. *s.f.*—sphenopalatine foramen. *s.g.*—sphenopalatine ganglion. *s.n.*—sphenopalatine nerves.

That the two types of respiratory response derived from the pharyngopalatine mucosa involve separate and distinct nervous mechanisms will be pointed out in the discussion of the nerve extirpation experiments.

The palatine accelerator respiratory reflex. The Vidian nerve (nerve in the pterygoid canal). The palatine accelerator respiratory reflex is mediated by the Vidian nerve, the afferent nature of which has been demonstrated by Yagita (1914), Rhinehart (1918–19), Fenton and Larsell (1928) (1928a), Blier (1930), and Blier and Luckhart (1931). That the afferent fibers of the Vidian nerve are derived from the greater superficial petrosal nerve is agreed upon by all of the above mentioned authors.

To facilitate the description of our operative procedure with reference to the Vidian and maxillary nerves, figure 1 has been incorporated in the text. A pharyngeal branch of the sphenopalatine ganglion was not found, a fact also reported by Rhinehart (1918–19) for the mouse.

While section of the Vidian nerves (fig. 1, *n.p.c.*, nerve in pterygoid canal) alone does not abolish the accelerator respiratory response resulting from mechanical stimulation of the pharyngopalatine mucosa as a whole, it does abolish the respiratory acceleration that is derived from the mucosa of the soft palate. In place of the original respiratory acceleration, one obtains complete inhibition of respiration from the soft palate.

In order to follow the pathway involved in the palatine accelerator reflex more peripherally, the anterior poles of both sphenopalatine ganglia (fig. 1, *s.g.*) were cut across, thereby severing the lesser palatine nerves (fig. 1, *l.p.n.*) which can be traced to the soft palate. This operation abolished the accelerator response. Inhibition resulted instead.

The afferent pathway for the accelerator respiratory reflex of the soft palate therefore involves the lesser palatine nerve, sphenopalatine ganglion, Vidian nerve, greater superficial petrosal nerve, and nervus intermedius. This conclusion finds support in the fact that electrical stimulation of the Vidian nerve causes marked acceleration in respiration. Blier (1930) and Blier and Luckhardt (1931) have made a similar observation in the dog.

That the impulses traversing the Vidian nerve follow the course described above, and not the deep petrosal nerve, internal carotid nerve, and cervical sympathetic trunk, is supported by the fact that respiratory acceleration is elicitable from the soft palate even after the cervical sympathetic trunks are cut. Also electrical stimulation of the central end of the cervical sympathetic trunk has absolutely no effect on respiration, an observation also reported by Ranson and Billingsley (1918) and Cleveland (1932). These authors believe that there are no afferent fibers in the internal carotid nerve, thus precluding the possibility of the Vidian nerve receiving any afferent fibers from the cervical sympathetic trunk through the deep petrosal nerve.

The palatine inhibitory respiratory reflex. The maxillary division of the trigeminal nerve. The inhibitory respiratory response which results from mechanical stimulation of the soft palate after section of the Vidian nerves, is abolished by section of the greater palatine branches (fig. 1, *g.p.n.*) of the maxillary nerve.

The pharyngeal accelerator respiratory reflex. The glossopharyngeal nerve. While section of the glossopharyngeal nerves does not abolish the accelerator respiratory response resulting from mechanical stimulation of the pharyngopalatine mucosa as a whole, it does abolish the accelerator response elicitable from the pharyngeal mucosa. A strictly homolateral

distribution of the glossopharyngeal nerves to the pharyngeal mucosa is present in some cases.

While neither the Vidian nor glossopharyngeal nerves, when cut alone, abolish the accelerator respiratory response derived from the pharyngopalatine mucosa, section of both of these nerves bilaterally has a nullifying effect on this reflex. Following the abolition of the accelerator respiratory reflex, one obtains respiratory inhibition as the result of mechanical stimulation of the pharyngopalatine mucosa. This inhibitory response is comparable to that obtained two minutes after the application of cocaine to the pharyngopalatine mucosa. In one case the inhibitory response became evident after the afferent fibers mediating the predominant accelerator response had been cut, in the other case the same afferent fibers were made non-functional by the anesthetic effects of cocaine.

The rôle of the glossopharyngeal nerve in mediating the accelerator respiratory response of the pharynx is supported by the observation of Teitelbaum and Ries (1935) that electrical stimulation of the central end of the glossopharyngeal nerve usually causes respiratory acceleration in the cat.

The pharyngeal inhibitory respiratory reflex. The vagus nerve. In a number of experiments bilateral vagotomy superior to the ganglion nodosum was found to be effective in abolishing the pharyngeal inhibitory respiratory reflex which resulted after abolition of the pharyngeal accelerator reflex. Instead of inhibition, we merely obtained a more or less marked increase in the amplitude of the respiratory excursion postoperatively. A similar effect was obtained after section of the pharyngeal branches of both vagus nerves. In one cat there was evidence that the superior laryngeal nerves also mediated a minor portion of the pharyngeal inhibitory reflex.

While Knoll (1882) reported that stimulation of the central end of the pharyngeal branch of the vagus in the rabbit does not affect respiration, this branch definitely inhibits respiration in the cat, thus confirming its rôle in mediating the pharyngeal inhibitory respiratory reflex.

Discussion. The observations reported above provide further support for the anatomically established concept with regard to the segmental distribution of visceral nerves. The palate is an outgrowth of the first or mandibular arch which is innervated by the greater superficial petrosal and maxillary nerves. These same nerves make up a functional pair which mediates antagonistic respiratory reflexes from the palatine mucosa. The pharynx is derived from the third visceral arch which is innervated by the glossopharyngeal and vagus nerves. These two nerves control the respiratory reflexes elicitable from the pharyngeal mucosa. The following table summarizes our results and indicates the segmental distribution of the nerves involved.

BRANCHIAL ARCH	DERIVATIVE OF ARCH	NERVE SUPPLY	
		Accelerator respiratory reflex	Inhibitory respiratory reflex
Mandibular arch	Palate	Greater superficial petrosal nerve	Maxillary nerve
Third branchial arch	Pharynx	Glossopharyngeal nerve	Vagus nerve

The antagonistic nature of the respiratory responses mediated by the individual nerves supplying the palatine and pharyngeal regions respectively, is in harmony with the data pertaining to the reflexes elicitable from the carotid sinus. Not only is the carotid sinus capable of producing pressor and depressor vasomotor effects, but it can also accelerate or inhibit respiration reflexly. It is therefore evident that respiratory reflexes, as in the case of vasomotor reflexes, involve a dual afferent nervous mechanism, one component of which controls respiratory acceleration, and the other controls respiratory inhibition.

SUMMARY

1. Mechanical stimulation of the pharyngopalatine mucosa of the cat causes in most cases acceleration of respiration. In some instances an inhibitory response is also normally present.

2. Cocaine applied to the pharyngopalatine mucosa abolishes first the hyperpneic response, and then the inhibitory response.

3. The palatine accelerator respiratory reflex is mediated by the greater superficial petrosal branch of the nervus intermedius. The palatine inhibitory reflex is mediated by the maxillary division of the trigeminal nerve.

4. The pharyngeal accelerator respiratory reflex is mediated by the glossopharyngeal nerves. The pharyngeal inhibitory reflex is mediated by the pharyngeal branch of the vagus nerve, and sometimes by the superior laryngeal nerve also.

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DISTENTION, A STIMULUS FOR UTERINE GROWTH IN UNTREATED, OVARIECTOMIZED RABBITS[1,2]

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That distention may be an adequate stimulus for uterine growth is suggested by the fact that the tissues of gravid uterine cornua in unilateral pregnancy in rabbits (Knaus, 1930; Hammond, 1935; Markee, Wells and Hinsey, 1936) and cats (Markee and Hinsey, 1935) are larger than are the tissues of the non-gravid horn which is subjected to the same hormonal conditions. Still more suggestive is the fact that when rolled rubber dam is inserted into a uterine cornu during pseudopregnancy in the rabbit, an increase in the rate of growth occurs in this distended cornu as compared with the non-distended cornu (van Dyke and Gustavson, 1929). Finally, Markee, Wells and Hinsey (1936) have described growth in uteri of normal rabbits with ovaries intact when the uteri are distended with fluid secreted by their own endometrial glands.

In the following experiments a dissociation of the effect of distention from the simultaneous action of the ovarian hormones is achieved by the insertion of paraffin pellets of known size into the uteri of ovariectomized rabbits. A comparison of the size and structure of the distention sites so produced with non-distended and acutely distended sites is thus available. In addition, data from such comparisons enable one to correlate the growth response of the uterus with the degree of stretching to which the uterus is subjected (i.e., intensity of stimulation).

MATERIALS AND METHODS. Eleven mature rabbits of mixed stock were used. Ovariectomy was performed on the seventh day of pseudopregnancy following the injection of human urine of pregnancy. As a consequence of this precaution, the uteri of the various animals were in a comparable hormonal state at the time of the first operation. One week after ovariectomy, greased paraffin pellets (m. p. 54°) of known size were inserted aseptically into the uterus. Two sizes of pellets were used ($\frac{1}{4}$ inch

[1] Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

[2] Preliminary report read before the Society for Experimental Biology and Medicine, February 19, 1936, at New York (see Reynolds and Kaminester, 1936).

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and $\frac{1}{8}$ inch in diameter). The pellets were about $\frac{3}{4}$ inch in length. Access to the uterus was made through the cervix after a small hole had been cut in the ventral aspect of the vagina. This procedure left the uterus uncut. In nine animals one pellet was inserted into one cornu; in two rabbits a pellet was inserted into each horn so that a total of thirteen observations was made. A loose ligature through the uterus at each end of the pellets served to anchor them in place, yet no fluid which may have been secreted by glands within the confined space was retained.

TABLE 1

Data on uterine growth in chronically distended uteri in ovariectomized rabbits

(Areas in square millimeters of average cross-sections)

RABBIT	$\frac{R_{ut}}{R_p}$	ENDOMETRIUM			MYOMETRIUM			WHOLE UTERUS (LESS LUMEN)		
		A Non-distended	B Chronic distention	C Per cent increase	A Non-distended	B Chronic distention	C Per cent increase	A Non-distended	B Chronic distention	C Per cent increase
½" *pellets*		*sq.mm.*	*sq.mm.*	(*net*)	*sq.mm.*	*sq.mm.*	(*net*)	*sq.mm.*	*sq.mm.*	(*net*)
R-6	0.81	4.9	12.5	156	11.5	30.4	164	16.4	42.9	161
R-10	0.74	2.7	6.8	152	11.6	27.0	132	14.3	33.8	136
R-8	0.72	1.9	4.8	152	8.8	19.4	120	10.7	24.2	126
R-9	0.83	5.4	14.3	165	12.8	25.3	97	18.3	39.6	116
R-7	0.68	3.5	6.6	88	7.3	15.6	113	10.8	22.2	106
R-28	0.66	3.8	4.6	22	8.1	13.9	71	11.9	18.5	56
R-12	1.40	6.6	6.3	0	17.1	27.9	58	23.6	34.2	44
¼" *pellets*										
R-5	1.13	3.5	8.5	143	4.9	11.4	132	8.4	19.9	137
R-1	1.45	3.8	5.0	32	10.9	17.0	56	14.7	22.0	49
R-3	1.68	5.6	7.1	27	14.8	18.2	23	20.4	25.3	24
R-27	1.49	4.5	5.7	26	11.5	14.7	28	16.1	20.5	27
R-27	1.49	4.5	5.1	13	11.5	14.8	28	16.1	20.0	24
R-28	1.45	3.8	4.4	15	8.1	11.4	40	11.9	15.8	32

Two weeks were permitted to elapse, at the expiration of which time three segments of uteri were removed from each animal and fixed in formol-acetic acid fixing solution. These segments were 1, the site of chronic distention; 2, a non-distended portion of the uterus, and 3, a site into which a pellet had been inserted just prior to removal of the tissue (acute distention). The distended tissues were fixed with the pellets inside them. Pellets used for segments 1 and 3 were cast in the same mold and were therefore of equal diameters except as noted in table 1. The tissues were then cut at ten micra, stained with hemotoxylin and eosin and utilized in the histological studies and measurements described below.

RESULTS. A. *Comparative histology of chronically distended, acutely*

distended and non-distended uteri. Non-distended uterus at the end of three weeks of castration. (Fig. 1 A.) *Lumen:* collapsed. There is no secretion or exudate present. *Endometrium:* Lining epithelium is low columnar in type, the cells are not particularly active. The nuclei are large, vesicular and occupy a large portion of the cell. Gland spaces are either absent or appear but infrequently and are limited to the superficial portion of the endometrium. The lining cells are low columnar in type and not active. The nuclei are compact and not especially active. The stroma is compact in the superficial portion. Vascularity is not prominent. *Myometrium:* The cells are compactly arranged, nuclei are prominent and occupy a large portion of the cell. Vascularity is not prominent in the vascular zone between the circular and longitudinal muscle coats.

Acutely distended uterus of the castrated rabbit. (Fig. 1 B.) *Lumen:* widely distended. There is no secretion or exudate present. *Endometrium:* In some sections, the endometrium is lacking around a portion of the circumference. Where present, the lining epithelium is flattened out. Toward the free side, the stroma is either lacking entirely so that the endometrial lining rests directly on the muscle or is reduced to a few cells. On the mesometrial side, some stroma persists. It is compact and almost devoid of vascularity. No hemorrhage is visible. *Myometrium:* The circular layer shows cells to be compactly arranged and bearing evidence of stretching. The nuclei are small and compact. The longitudinal layer shows little departure from the normal. No hemorrhage is seen in the vascular zone.

Chronically distended uterus of the castrated rabbit. (Fig. 1 C.) *Lumen:* widely distended. No exudate or secretion present. *Endometrium:* The lining epithelial layer is columnar in type. The cells are large, the nuclei large and vesicular. The stroma near the lumen is compact. The cells here are larger than in the non-distended uterus. In the deeper portion there is moderate edema. The glands are usually lacking. When present, they are represented by shallow depressions in the stroma. They are lined by a single layer of tall columnar epithelium. Vascularity is more pronounced than in the non-distended or acutely distended uteri but nowhere may it be said that there is true congestion. No evidence of inflammation is present. *Myometrium:* The circular layer shows marked hypertrophy of the individual fibers. Cytoplasm is increased in amount. Nuclei are larger and more vesicular than in the non-distended or acutely distended uteri. In the longitudinal layer, there is moderate edema. In the vascular layer, there is considerable increase in the number and size of the vessels.

Summary. From the above account it is clear that chronic distention of the uterus effects hypertrophy of both the myometrium and endometrium without associated irritative changes. It is also clear that these

growing uteri are unlike uteri at the time of oestrus, particularly as regards the freedom from congestion and the lack of many active epithelial glands. The extent of increase in the various layers can be appreciated best from a study of the percentage increases (table 1) taking place in each case. The contrast is also well shown in figure 1. It is possible that there is hyperplasia as well as hypertrophy of the muscle coat, but the exact determination of this is not attempted at the present time.

B. *Functional aspects of chronic uterine distention. Method of analysis.* Estimation of the amount of growth was made by projecting many cross sections of the various tissues and calculating from drawings of these, with the aid of a planimeter, the area of endometrium, myometrium, and entire uterus (less lumen) respectively in an average cross section. By correcting for the magnification (12.14x), the actual areas were obtained

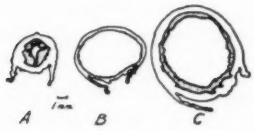

Fig. 1

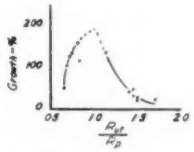

Fig. 2

Fig. 1. Drawings of projections of representative cross sections of uterus from rabbit R-6 at the end of third week of ovariectomy. Endometrial areas (stippling) and myometrial areas (clear). A, non-distended; B, acutely distended; C, chronically distended for two weeks. Same magnification. See table 1 for percentage increase of C over A for this and other rabbits.

Fig. 2. Chart showing the effect of distention on the distention growth-response, expressed in percentage increase of normal (non-distended) uterus. Points based on data from table 1 for growth of whole uterus, less lumen.

(table 1). Some degree of arbitrary judgment was necessarily exercised in delimiting the myometrium in the region of the mesometrium, but inasmuch as this was done by one individual who used uniform technic, any error due to such variation may be considered constant. Artifacts occurring in fixation were considered uniform for all the tissues and hence no allowance was made for them. Very careful and extensive use of this "cross sectional" method of analysis by Markee, Wells, Hinsey (1936) has shown that the amounts of the component tissues of left and right cornua in normal rabbits are uniform and nearly the same in amount.

Distention-factor (intensity of stimulation). A correlation was made of the degree of distention and the amount of growth taking place. The amount of stretching to which the uterus is subjected is expressed as the ratio between the sizes of the undistended uterus (including lumen) and the distending pellets. This was done in the following manner:

The total area of a cross section of the non-distended uterus, including the lumen, was determined. Such an area has a very irregular shape. It was regarded as a circle, however, the radius of which served as an index to the size of the undistended uterus. The radii of the pellets were known. The factor of distention is, therefore,

$$\frac{\text{radius of the undistended uterus (including lumen)}}{\text{radius of the distending pellet}}$$

or

$$\frac{\text{R ut}}{\text{R p}}.$$

Table 1, column 2, shows that this ratio varied in our experiments from 0.66 to 1.68. In other words, the size of the uterus was approximately two-thirds the size of the quarter-inch pellets at the one extreme and somewhat over one and one-half times the size of the eighth-inch pellets at the other.

Expression of the growth response. In table 1, the cross sectional areas of endometria, myometria and of both combined in each case are expressed in terms of square millimeters, both for the non-distended and chronically distended uteri (columns A and B). In the third column (C) in each group, the difference is represented as a percentage increase, the area of the non-distended cross sections being regarded as 100 per cent.

Correlation of the amount of growth of the chronically distended uterus with the amount of distention. (Fig. 2.) In this graph the percentage increase in growth of the chronically distended portion of uterus is plotted against the $\frac{\text{R ut}}{\text{Rp}}$ ratio. It will be seen that when the ratio of the size of the undistended uterus to the size of the distending pellet approaches 1:2, the pellet is becoming a less adequate stimulus for growth. Similarly, when the ratio approaches 2:1, the pellet is a less adequate growth stimulus. The point of optimum growth is found to be when the ratio is approximately 1:1. Inspection of the percentage increase in myometrium and endometrium in each case shows that a fairly close agreement is obtained between them. Thus when the percentage increase in myometrium is high, the endometrial increase is usually high; similarly, when the amount of growth of myometrium is small, that of the endometrium is small. The new growth is roughly proportional, therefore, for the two types of tissue.

CONCLUSIONS

1. Uterine growth may be induced in ovariectomized rabbits by means of chronic intrauterine distention.

2. The percentage increase in endometrium and myometrium is about equal.

3. The endometrium increases by active proliferation of the epithelium as well as hypertrophy of its cells and an increase in the amount of stromal connective tissue. There is no restitution of epithelial glands.

4. The myometrium undergoes marked hypertrophy, and possibly hyperplasia. The increased amount of cytoplasmic to nuclear material in these cells in chronically distended uteri is striking.

5. The changes observed are not the result of inflammation or irritation.

6. The form of the growth curve of the uterus of the ovariectomized rabbit under the stimulus of chronic distention for a period of two weeks is indicated. The curve shows that to be an effective stimulus the distending influence must be somewhat greater than one-half and somewhat less than twice the size of the undistended uterus. A maximal growth stimulus is found to be when the distending influence is approximately equal to the size of the undistended uterus.

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EFFECT OF RESTRICTION OF INORGANIC SALTS IN THE DIET ON ORGAN GROWTH[1]

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Previous studies have shown that strict limitation of mineral salts in the diet of the albino rat results in cessation of growth, alteration of body form and changes in the composition of tissues and blood. Certain well-defined adjustments in the mineral and protein metabolism to this nutritional deficiency appear to be important in maintaining the life of the animal. A further approach to the complete understanding of the compensatory changes in the organism to the deficiency in salts can be made by determining the development of vital organs under this condition. A recent report (Swanson, Storvick and Smith, 1936) indicates the widespread involvement of the kidney in the adjustment to the lack of salts. The present paper deals with the changes in the spleen, adrenals, testes and heart and with the relation of some of the observations to important biological processes.

PLAN OF EXPERIMENT. Four groups of 24 animals each were used: group I, normal animals of the same age as the experimental rats received *ad libitum*, a synthetic ration adequate in all known respects; group II was given a diet providing the same quantity of protein, vitamins and salts as group I, but limited in energy intake to that ingested by group III; group III, the experimental animals, fed in unrestricted quantity a ration poor in inorganic constituents; and group IV, the weight or physiological controls, a series of normal animals of the same average body weight as the experimental group.

The composition of the diets fed to these respective groups is given in

[1] The data reported in this paper are taken from a dissertation presented by Pearl P. Swanson in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1930.

A preliminary report on some of the findings was presented before the American Institute of Nutrition at New York, March, 1934.

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[2] Alexander Brown Coxe Fellow, Yale University, 1929–30.

table 1. The experimental rations were first offered to the rats of each group when a body weight of 120 grams was attained.

At the end of the experimental period (90 days), the animals composing groups I, II and III were stunned and killed by bleeding from the abdominal aorta after which the organs were removed. Group IV was similarly treated when the animals had attained the desired weight.

TABLE 1

Experimental diets

	per cent
Adequate ration:	
Casein*	18
Dextrin	51
Hydrogenated fat†	27
Salts‡	4
Calories per gram, 5.2	
Restricted-intake ration:	
Casein*	25 7
Dextrin	41 6
Hydrogenated fat†	27 0
Salts‡	5 7
Calories per gram, 5.1	
Low-salt ration:	
Casein*	18
Dextrin	55
Hydrogenated fat†	27
Salts‡	0
Calories per gram, 5.4	

* Poor in ash.

† Crisco.

‡ Osborne, T. B. and L. B. Mendel, J. Biol. Chem. **32:** 309, 1917.

Vitamin adjuvants to all rations:

Yeast, 200 mgm.
Alcoholic extract of wheat germ, 1 cc.
Wheat germ oil, 3 drops
Cod liver oil, 5 drops.

In addition to the groups already enumerated, other animals, 93 in number, were divided into two groups. Some of these were killed at once (body weight, 120 grams) so as to obtain the average picture at the beginning of the experiment. The other members of each group were fed the adequate and salt-poor rations, respectively, and were then sacrificed after 21 days, 42 days, and 63 days of experimental feeding. The procedure made possible the measurement of changes occurring in the course of the experiment that were not apparent in the final determinations. The

organs—heart, testes, spleen, and adrenals—were removed, trimmed of adhering tissue, and drained of blood.

RESULTS. The indispensability of the inorganic constituents in the diet is emphasized by the significant changes observed in every organ studied when a diet deficient only in mineral salts was fed.

Growth of heart. The average weight of the hearts of rats which had subsisted upon normal, restricted normal, and low-salt diets for a period of 90 days is given in table 2. The average weight of the hearts of the weight control rats is also recorded here. The data show that the weight of the heart was definitely low in animals fed a diet in which the inorganic elements were strictly limited. It was some 41 per cent smaller than the heart of the age control $\left(\frac{d.}{p.e.d.}, 27\right)$[3] and 9 per cent smaller than the heart

TABLE 2

Weight of certain organs after 90 days of experimental feeding

ORGAN	WEIGHT CONTROLS			AGE CONTROLS (FOOD AD LIBITUM)			CALORIE CONTROLS (ENERGY RESTRICTED)			EXPERIMENTAL ANIMALS (LOW-SALT FOOD)		
	Number of rats	Average weight	Standard deviation	Number of rats	Average weight	Standard deviation	Number of rats	Average weight	Standard deviation	Number of rats	Average weight	Standard deviation
		grams	*grams*		*grams*	*grams*		*grams*	*grams*		*grams*	*grams*
Heart	33	0.639	0.058	24	0.994	0.092	24	0.714	0.061	24	0.581	0.065
Testes	24	2.005	0.198	16	2.699	0.065	24	2.706	0.033	24	2.096	0.046
Spleen	24	0.632	0.361	16	0.640	0.152	24	0.432	0.075	24	0.297	0.103
Adrenals	20	0.037	0.004	12	0.050	0.006	23	0.040	0.007	18	0.042	0.009

of a normal animal of the same size $\left(\frac{d.}{p.e.d.}, 5.2\right)$. The difference between the average heart weight of these rats and that of the calorie controls was also highly significant $\left(\frac{d.}{p.e.d.}, 11\right)$. A decreased heart weight following this specific dietary deficiency is not consistent with the findings of Winters, Smith, and Mendel (1927), who, working with newly-weaned rats, reported a significant gain in the weight of the heart of animals subjected to a similar diet for 40 days. However, since the heart of the young

[3] In the ratio, $\frac{d.}{p.e.d.}$, is the difference between the means and *p.e.d.* is the probable error of the mean difference. If the value of the ratio is equal to 3.0, the difference in the two means may be considered significant, i.e., the chances are greater than 20 to 1 that the difference is real.

animal undergoes a very rapid phase of development (Donaldson, 1924), the growth impulse may have overshadowed the inhibitory effect of the diet when the defective ration was offered to newly-weaned rats. In the present experiment, the only increment in heart weight occurred in the first 21 days of the period (table 3). Thereafter, no significant differences were found between the mean weights of hearts removed at successive experimental periods. Increments correlated with changing body weight (Donaldson, 1924) were observed at each interval in the normally growing control animals.

TABLE 3

Progressive changes in the weight of certain organs of rats maintained upon a normal adequate diet and upon a ration poor in salts

INTERVAL OF EXPERIMENT	HEART			TESTES			SPLEEN			ADRENALS		
	Number of determinations	Average	Standard deviation	Number of determinations	Average	Standard deviation	Number of determinations	Average	Standard deviation	Number of determinations	Average	Standard deviation
		grams	*grams*		*grams*	*grams*		*grams*	*grams*		*grams*	*grams*
Group I. Normal rats fed adequate diet												
Beginning	12	0.480	0.057	12	1.265	0.292	12	0.395	0.151	8	0.034	
After 21 days	13	0.634	0.047	13	2.092	0.195	13	0.478	0.256	5	0.036	
After 42 days	8	0.833	0.102	8	2.971	0.407	8	0.677	0.161	9	0.046	0.008
After 63 days	13	0.832	0.066	13	2.784	0.262	13	0.550	0.183	13	0.035	0.006
After 90 days	24	0.994	0.092	16	2.699	0.367	16	0.640	0.152	12	0.050	0.006
Group II. Experimental rats fed low-salt diet												
Beginning	12	0.480	0.057	12	1.265	0.292	12	0.395	0.151	8	0.034	
After 21 days	14	0.593	0.055	14	2.267	0.255	13	0.457	0.167	14	0.037	0.005
After 42 days	14	0.580	0.057	12	2.468	0.180	12	0.349	0.085	12	0.053	0.006
After 63 days	10	0.614	0.102	8	2.019	0.354	10	0.323	0.050	10	0.040	0.007
After 90 days	24	0.581	0.063	24	2.096	0.337	24	0.297	0.103	18	0.042	0.009

Growth of testes. The data presented in table 3 illustrate the marked development of the testes of normal rats in the early days of the experiment. The increase in weight was approximately 0.800 gram in each of the first two intervals. These increments apparently occurred at the time of gonadal and pubertal development. Thereafter, there was a normal regression in the weight of the testes probably marking the stabilization of maturity. If testes weight is plotted against body weight, the graph does not take the same form as a similar curve based on data obtained from normal rats presented by Donaldson (1924). In the present instance,

the testes were much heavier in relation to body weight and furthermore, the characteristic regression in weight is not apparent from Donaldson's data.

This organ in the low-salt rat was significantly smaller at the termination of the experiment as shown by comparisons with either age control, and only slightly larger $\left(\frac{d.}{p.e.d.}, 1.7\right)$ than its normal weight control (table 2). It is not to be regarded as an infantile testis, however, for it showed more than normal growth for a time after which a shrinkage occurred (table 3). Histologic examination indicated that in spite of the definite regression in weight that took place during the test period, the gland was entirely normal in cell structure at the termination of the experiment.[4]

It should be emphasized that the changes observed were the result of an uncomplicated dietary deficiency for vitamin E was supplied in the ration. Many of the observations in the literature relating to the effect of specific nutritional inadequacies upon gonadal development have been obscured by the fact that the anti-sterility vitamin was not included in the dietary mixture.

Growth of spleen. The paucity of dietary salts produces a spleen of subnormal size (table 2). At the end of the test, it was less than one-half as large as the organ of the weight control despite the fact that the size of the spleen is correlated with body weight (Donaldson, 1924). Table 3 shows that only during the first three weeks of the experiment was the increment in the size of the spleen of the animals fed the low-salt food approximately normal. A shrinkage of the organ, evident at the end of the second interval, became more pronounced as the experiment progressed. An inhibitory effect of the ration upon normal splenic development is, therefore, apparent.

Growth of adrenals. Complete or partial inanition induced by a reduction in calories (Jackson, 1915), by a change in the qualitative nature of a balanced salt mixture (Haag and Palmer, 1928), or by a deficiency of vitamin B (McCarrison, 1919) causes an enlargement of the adrenal gland. In the present experiment, the organ on the basis of absolute weight was small rather than large in relation to the normal picture when the only dietary adjustment consisted of a limitation of the energy needs of the animal (age controls vs. calorie controls, table 2).

The data in table 3 show that physiologic adjustments brought about by an absence of dietary salts are reflected in adrenal size. There was a marked growth during the first six weeks of the experiment. During this period, the normal organs increased 35 per cent in weight, the adrenals of the low-salt animal, 56 per cent. The augmentation in size occurred

[4] Personal communication from Dr. K. E. Mason.

even though the body weight of the rat was now essentially constant. However, following this initial growth a shrinkage in the size of the adrenal took place. In spite of the regression, the adrenal of the rat given the ash-poor food was larger than that of the normal control in all but the last period.

On the basis of findings in part substantiated by Gaunt, Tobin, and Gaunt (1935), Rubin and Krick (1933) have postulated that the action of adrenal hormone may be one of regulation of salt metabolism. If this is true, compensatory changes of the character noted in the first half of the experiment might be expected in the adrenal when the salt content of the ration is reduced to a minimum. Although the data are not entirely confirmatory, the work of different groups of investigators has suggested that animals with adrenal insufficiency are unable to regulate the water and electrolyte content of their tissue and plasma (Winter and Hartman, 1933; Ponder and Gaunt, 1934; Loeb, 1935). Evidence has also been obtained in the present series of investigations that the complete withdrawal of the inorganic constituents from the otherwise adequate experimental diet gives rise to a disturbance in the water metabolism. This has been shown by an abnormal distribution of water in certain tissues (Swanson and Smith, 1932a; Swanson, Storvick, and Smith, 1936), by a retardation of the processes of natural dehydration (Swanson and Smith, 1932b), and by an augmented water intake and urine output (Swanson, Timson, and Frazier, 1935). A hydrophilic condition of certain soft tissues, notably the liver and muscle, has likewise been reported after extirpation of adrenal glands (Silvette and Britton, 1933).

The changes in the water content of the tissues and in the weight of the adrenals of the animals used in the present experiment were, therefore, next compared. The percentage of water was determined in muscle removed from the thighs of the age control and of the experimental groups of rats already described at the beginning of the experiment and after 21, 42, 63 and 90 days of experimental feeding. One hundred and sixteen samples were analyzed. The water content of the bloods from the same animals has already been reported (Swanson and Smith, 1932b). The two sets of data present objective evidence of changes in tissue hydration induced by the low-salt feeding. The correlations between these findings and the respective weights of the adrenals are shown in table 4. At the termination of the experiment the organism fed the diet poor in salts was essentially hydrated. These findings are augmented by other data (Swanson, 1930; Swanson, Storvick, and Smith, 1936) which show that in addition to the blood and muscle, other tissues have an increased water content, i.e., the heart, bone, and kidney. Likewise, it is known (Light, et al. 1934) that the water content of the tissues composing the entire body of a rat fed the salt-poor diet for 90 days approximates that of an animal 57 days

old. After 42 days, a dehydration of muscle greater than that which could be attributed to advancing age (Moulton, 1923) occurred in the low-salt rats (table 4). The condition was reflected in a more hydremic blood. Associated with the dehydration of the muscle were changes in the fresh weight of the adrenal gland. During this period the adrenal of the rat fed the deficient diet increased in weight by 56 per cent whereas the change in the normal organ was only 35 per cent. That this is a reverse compensatory enlargement to meet shifts in the distribution of water in the tissues induced by the paucity of dietary salts is in accord with the general belief that tissue dehydration follows adrenalectomy (Silvette and Britton, 1933; Loeb and co-workers, 1933; Swingle, et al. 1933; Harrop, et al. 1933; and Marine and Baumann, 1927). After six weeks, however, a progressive

TABLE 4

Correlation between changes in hydration of tissues and adrenal weights

INTERVAL OF EXPERIMENT	WATER IN MUSCLE		WATER IN BLOOD		WEIGHT OF ADRENAL		d./p.e.d.		
	Age-control animals	Low-salt animals	Age-control animals	Low-salt animals	Age-control animals	Low-salt animals	Muscle	Blood	Adrenal weight
	per cent	*per cent*	*per cent*	*per cent*	*grams*	*grams*			
Beginning of experiment	76.8	76.8	81.3	81.3	0.034	0.034			
After 21 days	75.6	74.8	80.6	80.0	0.036	0.037	3.7	2.6	0.0
After 42 days	72.6	71.0	79.7	80.6	0.046	0.053	1.8	2.6	3.4
After 63 days	75.5	75.1	79.8	81.9	0.035	0.040	3.1	4.1	2.6
After 90 days	74.3	75.3	79.4	81.0	0.050	0.042	3.0	4.4	4.4

shrinkage of the adrenal took place, concomitant with an increased and sustained water content of blood and muscle.

SUMMARY

A deficiency of inorganic materials in the diet manifests itself in two ways on the development of the organs. First, a short period of subnormal growth may occur that is followed by a distinct regression in weight. As a result, the organs may weigh less at the end of the experiment than do the same members in normal rats of similar body weight. The heart and spleen are examples of organs thus affected. Second, there are those organs whose rate of growth is at first abnormally accelerated by the absence of dietary salts but which later undergo a shrinkage. Of these, the kidney (Swanson, Storvick and Smith, 1936), the testes, and the adrenals are examples. The testes show a loss in weight by the time the experiment is concluded. In spite of the shrinkage that occurs, the kidneys and adrenals are larger than the same organs in normal animals of equal size.

The possible relation of adrenal hormone to the regulation of salt metabolism has been pointed out on the basis of the correlation of changes in size of the adrenal and shifts in the distribution of water in blood and muscle.

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THE COCHLEAR RESPONSE AS AN INDEX TO HEARING[1]

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Experimental studies have in recent years yielded a wealth of information concerning the electrical activity of the cochlea and the action potentials of the auditory nerve. Many of the results of such investigations have been reported by Davis and his collaborators and have been adequately reviewed by Davis (1935). The apparatus for eliciting the responses has been briefly described in a previous publication (Black and Covell, 1936). A more complete description of a similar apparatus has been given by Garceau and Davis (1934).

A great deal of effort has been directed towards ascertaining the value of the cochlear response as an index to hearing. For this purpose a properly calibrated apparatus was found to be essential since it was necessary to obtain accurate results of the order expected in engineering acoustics. Without the use of such equipment for the testing of the cochlear response confusion might easily arise regarding the interpretation of results. An example of such a predicament would be the differentiation between sensation and loudness levels when they closely approximate each other.

The purpose of this investigation is to sufficiently determine the nature of the cochlear response so that the studies may be extended to hearing problems related to the influences of drugs (Covell, 1936), toxins, dietary and other factors.

RESULTS. The results were averaged from measurements of the responses from the ears of twelve cats. Measurements were made on many ears but only those giving consistent and steady responses were selected for study. None of the ears selected revealed any pathologic change, but certain of those remaining revealed changes which will be correlated with the findings in a subsequent contribution.

In figure 1 the cochlear responses are plotted on a logarithmic scale. A straight line represents the relation between the logarithm of the response and the logarithm of the stimulus for a considerable range of stimuli.

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Below one microvolt the relationship has not been determined because of the limitations imposed by the measuring apparatus. It is evident that the relation between the response E, in microvolts and the strength of the stimulus, P, in microwatts, for the straight part of the curves, is expressed by an equation: $E = CP^n$, where C and n are constants for any one frequency.

The curve of the 400 cycle response is nearly a straight line from -55 db to 0 db and the slope of this line is such that a stimulus change of 1:100, (20 db) corresponds to a voltage change of 1:10. The equation is, therefore, given by the square root law:

$$E = \left(\frac{P}{P_e = 1}\right)^{1/2};$$

$Pe = 1$ is the power in microwatts required to give a response of one microvolt for the frequency under consideration. For this frequency there is, then, a linear relation between the acoustic pressure and the response voltage over a considerable range of stimuli.

The response for any frequency is equated:

$$E = \left(\frac{P}{P_e = 1}\right)^n$$

The values for n measured from the curves are:

Frequency	100	200	400	1,000	4,000	10,000
n	0.52	0.51	0.50	0.46	0.465	0.48

These data indicate that a given increment of stimulus causes an increment of response which is greater at low frequencies than at high frequencies. The results were directly checked by comparing them with the necessary change in stimulus to produce a specific change in response for various frequencies. The significance of this will be discussed later.

For the curves of figure 1 there is a linear relationship up to an output of 150 microvolts. The departure from this linear relation at high intensities of stimulation resembles the departure noted when any mechanical device is overloaded.

The smallest intensity level increment that can be subjectively detected is about 0.25 db. This fluctuation corresponds to approximately a 2.5 per cent change in the cochlear response. The change in the magnitude of the response is continuous with change of intensity. No step-like increments of response were detectable.

Maximum cochlear response. The average maximum response is plotted as a function of the frequency in figure 2. For each animal tested, the maximum values were measured directly by increasing the stimulus until

the plateau of the curve was reached for each test frequency. The stimulus necessary to give the maximum response is plotted in the same figure. The shape of this curve approximately resembles the threshold of feeling curve determined by Wegel (1932). The latter curve is also given, for comparison, in figure 2. It will be seen that the maximum response at 1000 cycles is reached for a stimulus 18 db below the human threshold of feeling. This was checked by having several observers place the tube used for testing the cats in their own external canals and the stimulus was increased until discomfort was reached. The results suggest that the threshold of

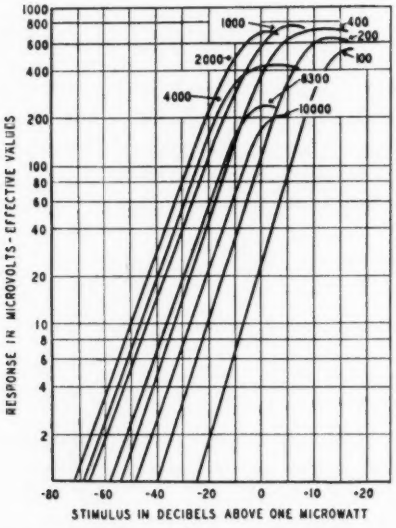

Fig. 1. The relation between cochlear response and stimulus. Recorded from the round window. (Average of twelve ears.)

feeling occurs at a lower stimulus for a cat than for a human. Since the comparison was made for equal energies delivered to the ear drum, some of this difference, but certainly not more than 3 db can be accounted for by the difference in ear drum areas.

Equal output curves of the cochlear response. From the data represented in figure 1, curves of equal outputs as a function of frequency were plotted and are shown in figure 3. Although the one microvolt curve is about 20 db above the human threshold of audibility, it was not difficult to hear the response in the headphones (connected to the amplifier output) for human threshold intensities of stimulation. The shape of the one microvolt

curve fits very closely the threshold curve of a cat as determined by Davis (1934).

The equal output curves resemble the contour lines of equal sensation levels. The rate at which they approach each other at low frequencies is

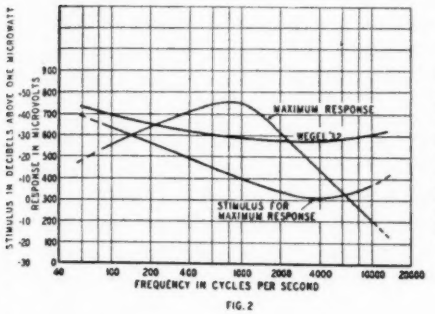

FIG. 2

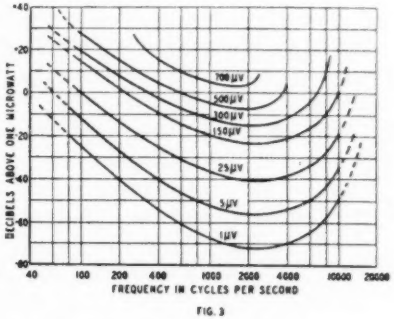

FIG. 3

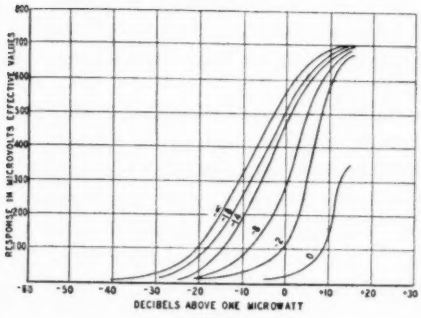

FIG. 4

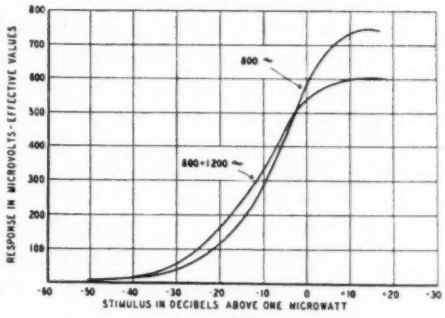

FIG. 5

Fig. 2. The maximum cochlear response and corresponding stimulus. (Average of twelve ears.) Wegel's (1932) threshold of feeling curve.

Fig. 3. Equal cochlear response contours. (Average of twelve ears.)

Fig. 4. The cochlear response to a 700 cycle tone measured in the presence of a 2000 cycle tone. The figures on each curve indicate the intensity of the 2000 cycle tone in db above one microwatt.

Fig. 5. The cochlear response to an 800 cycle tone and the total response to two tones of 800 and 1200 cycles per second. The abscissae show the intensity of the 800 cycle tone. The intensity of the 1200 cycle tone was 4 db less than that of the 800 cycle tone.

far less than that of equal loudness contours. An intensity change of 10 db at the one microvolt response level produces a change in the cochlear response of 9.2 db at 1000 cycles and 10.4 db at 100 cycles. The same change of intensity produces a 10 db change of loudness at 1000 cycles and a 17 db change of loudness at 1000 cycles (Fletcher, 1929). In other

words, loudness increases at a greater rate than does the cochlear response to tones of low pitch.

It has been pointed out by Davis (1935) that the curve of human audibility falls below the threshold of the electrical response at low frequencies. To account for this apparent discrepancy he points out that the apical region, where presumably the low tones are generated, is farthest from the recording electrode. Our observations have shown that equal response levels at various frequencies do not mean equal loudness and that the two are not identical.

The effect of one tone upon the response of another. It is a well-known fact that the presence of a loud tone decreases the ability of the ear to hear other tones. Curves of the cochlear response to a tone of 700 cycles measured in the presence of an interfering tone of 2000 cycles are shown in figure 4. The intensity of the interfering tone is indicated on each curve. The cochlear response was measured through a three section 1000 cycle low-pass filter and included only the response due to the 700 cycle stimulus. Tests made by replacing the output from the animal's ear by the output from a crystal microphone definitely proved that the decrease of the response to one tone caused by the presence of another tone took place in the animal's ear and not in the apparatus.

It was thought that a study of the relative decrease caused by various interfering frequencies would reveal some information concerning "masking." However, the investigation showed that the amount of decrease of the response to one tone caused by the presence of another tone (the interfering tone) was determined by the intensity (of the interfering tone) and was independent of the frequency of the latter. There was no correspondence to subjective masking. High frequency tones did not have a masking effect that was different from that of low frequencies. Interfering tones of intensities corresponding to equal response levels caused equal interference. The amount of decrease was small unless the interfering tone was of high intensity.

This effect is closely related to the maximum value of the cochlear response, which in turn may be related to the total number of nerve endings excited by a strong stimulus. This belief is strengthened by a consideration of the total cochlear response due to two tones.

Figure 5 shows the total response to two tones of 800 and 1200 cycles per second. In the same figure is shown the response to an 800 cycle tone. The abscissae show the intensity of the 800 cycle tone. The intensity of the 1200 cycle tone was 4 db less than that of the 800 cycle tone. These intensities corresponded to equal response levels. The output was measured on a thermocouple type meter and was the effective root mean square value. The root mean square value of output is given by the equation:

$$E = (E_1^2 + E_2^2)^{1/2}$$

Where, E_1 = effective value of response due to tone number one in the presence of tone number two.

E_2 = effective value of response due to tone number two in the presence of tone number one.

If, $E_1 = E_{2_1/_2}$ (the case in this test)

$E = (2)\, E_1 = 1.41\, E_1$

It was noted that for low intensities the total response to two tones was equal to 1.41 times the response to a single tone. It is therefore obvious that tones of low intensities do not interfere with each other. The effective maximum response to two tones was less than that to a single tone.

The total electrical response due to two tones having frequencies of 250 and 3000 cycles never exceeded the maximum response of the 250 cycle tone alone. The maximum cochlear response may be proportional to the total number of sensory cells excited and for high intensities a considerable portion of the basilar membrane may be set into vibration. Two tones of high intensity, not greatly differing in frequency, would stimulate nearly the same group of sensory cells. Therefore, a total response greater than that due to a single tone would not be possible.

SUMMARY

The results indicate that the use of the cochlear response as an index of hearing is limited. Although it is certain that many of the characteristics of the ear are reflected in the cochlear response some of the important phenomena of hearing are not present.

It has been stated by Davis, Derbyshire, Lurie and Saul (1934) that the maximum response occurs at the intensity for which a human observer begins to experience tinnitus and discomfort. They suggest that this marks the threshold of feeling. The resemblance of the curve plotted in figure 2 to the threshold of feeling curve substantiates their view. The threshold of feeling for the cat is, however, below that of the human.

Since the equal response levels do not conform to equal loudness levels, the contour of the threshold of the cochlear response (which corresponds to equal minimum detectable voltages at various frequencies) does not give the contour of the hearing threshold. These results show no loudness characteristics in the cochlear response. It is obvious that loudness is not related in a simple manner to the cochlear response. Over a large range of intensities the logarithm of the cochlear response is linearly proportional to the logarithm of the stimulus. A change of loudness over this range bears the same relation to a change of the cochlear response as it does to a change of stimulus. The phenomenon of loudness must be centrally located or if it originates in the cochlea it is not apparent in the response.

Subjective masking characteristics are not found in the cochlear response. Masking has been ascribed by Davis (1935) to the mechanism of trans-

mission of nerve impulses. However, an interfering tone of high intensity decreases the cochlear response to any other tone. This effect is possibly due to the fact that the intense interfering tone stimulates the sensory cells that would normally be stimulated by the original tone. By the same reasoning, two tones differing but slightly in frequency would stimulate the same group of sensory cells at high intensities and the total maximum response would not exceed that of one of the tones.

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CALCIUM AND PROTEIN CHANGES IN SERUM DURING SLEEP AND REST WITHOUT SLEEP

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It has been frequently reported that there is a change in the total serum calcium during sleep and anesthesia. In 1924, Cloetta and Thomann (3) reported a drop in serum calcium during anesthesia. One year later, Heilig and Hoff (8), using psychopathic patients, demonstrated an increase during sleep, while Demole (4) found a decrease. Gollwitzer-Meier (7) was not able to demonstrate any significant change under this condition.

On the basis of his work with Thomann as well as that done by others, Cloetta postulated a passage of calcium from the blood stream into the tissues, including the brain, during sleep and anesthesia. This view was supported by Demole and Fischer (5, 6) but refuted by Bergren and Moberg (1) who obtained a state resembling sleep by the injection of calcium chloride, potassium chloride, and on occasion, by merely inserting the syringe needle into the appropriate region. In 1929 Cloetta and Fischer (2) repeated the work of Bergren and Moberg using a great number of substances. They corroborated Demole insofar as $CaCl_2$ injections were concerned, and found that KCl caused an excitation. They also reported that injections into adjacent regions had no effect and maintained that the result was specific for calcium in a delimited area of the brain.

All previous investigators had only measured changes in total serum calcium, and, as indicated above, their findings were not consistent. This investigation was undertaken to re-determine whether any changes in blood calcium actually occurred during sleep, with special reference to ionized calcium.

PROCEDURE. Human subjects and dogs were used in this study. Preceding each experiment, no food was taken for 12 and 24 hours respectively. In the human subjects, blood samples were taken before going to sleep and immediately upon awakening. These observations were controlled by having the same individuals lie awake without actually sleeping for an equal length of time. In the animal experimentation an exact criterion of sleep was lacking although the conditions were conducive to sleep. Hence, reference is made to these periods as "rest." The exercise preceding one

series of experiments was carried out by means of a treadmill operating at a very slow speed.

The chemical methods used were as follows: Clark-Collip modification of the Kramer-Tisdall for total serum calcium; macro-Kjeldahl for serum proteins; McLean-Hastings nomogram method for ionized calcium (10); and the Congo red indicator method for circulating blood volume.

RESULTS. In 7 subjects, on 7 experimental nights, 5 to 7 hours of sleep were found to cause a slight decrease in total serum calcium which, coupled with a greater decrease in serum proteins, resulted in a slight but consistent increase in the calcium ion concentration of the serum. Similar

TABLE 1

Calcium and protein changes in serum during sleep and rest without sleep

		HUMAN SUBJECTS			DOGS		
		I Effects of sleep (5–7 hours)	II Effects of lying awake (5–7 hours)	III Effect of sleep and rest without sleep (1½–2 hours)	IV Effect of rest (1–2 hours) after mild exercise (30–60 minutes)	V Effect of rest (1–2 hours) without initial exercise	VI Effect of prolonged rest (5–6 hours)
Grams per cent serum proteins	Normal	7.79 (7.17–8.39)	7.77 (6.81–8.58)	7.79 (6.97–8.84)	6.12 (5.40–6.51)	6.43 (5.76–7.35)	6.82 (6.44–7.35)
	Experimental	7.25 (6.64–7.58)	7.10 (6.31–7.87)	7.10 (6.13–8.30)	5.76 (5.14–6.21)	6.25 (5.00–7.14)	6.19 (5.84–6.65)
	% difference	−6.7	−8.6	−8.8	−5.7	−2.8	−9.2
Mgm. Ca per 100 cc. serum	Normal	10.11 (9.74–10.49)	10.05 (9.32–10.59)	10.50 (9.55–10.99)	10.68 (10.27–10.98)	10.47 (9.74–11.41)	10.52 (9.35–11.39)
	Experimental	9.87 (9.43–10.36)	9.98 (9.35–10.38)	10.10 (9.04–10.81)	10.50 (9.95–11.27)	10.49 (9.52–11.21)	10.16 (9.90–10.36)
	% difference	−2.3	−0.6	−4.6	−1.8	+0.5	−3.1
mM Ca^{++} per kgm. H_2O	Normal	1.11 (1.07–1.16)	1.11 (1.00–1.22)	1.18 (1.10–1.28)	1.38 (1.30–1.46)	1.32 (1.14–1.52)	1.27 (1.14–1.36)
	Experimental	1.16 (1.10–1.19)	1.18 (1.08–1.26)	1.18 (1.09–1.36)	1.39 (1.29–1.48)	1.35 (1.17–1.61)	1.31 (1.21–1.37)
	% difference	+4.0	+6.0	+0.1	+0.9	+2.1	+3.0

changes were found in the same subjects after they had remained awake in a recumbent position for an equal length of time. With a shorter period (1½ to 2 hours) of both sleep and rest without sleep, there was, in 11 experiments, a more marked decrease in total serum calcium, a decrease in serum proteins no greater than with an interval of 5 to 7 hours, and no change in the resultant calcium ion concentration. No differences were found when the experimental periods occurred in the afternoon.

One and one-half to 2 hours after the beginning of the experimental period there was a 10 per cent increase in plasma volume in both sleep and rest. At this time, red blood cell, hemoglobin and hematocrit determinations showed a decrease.

Ten experiments, using 5 animals, showed the effects of 1½ to 2 hours of rest after a 30 to 60 minute period of mild exercise to be a small decrease in both serum proteins and total serum calcium, with no change in the calcium ion concentration. Without the initial exercise period, no consistency was seen in the changes in serum proteins and serum calcium obtained after rest, in 13 experiments using 5 dogs. However, where an increase in serum proteins was found, it was accompanied by an increase in total serum calcium, and vice versa. Under these conditions, calcium ion concentration showed no change. When the animals (4 dogs, 6 expts.) rested for a longer period (5 to 6 hrs.), there was found a decrease in both serum proteins and serum calcium with, however, a small, but consistent, increase in the calcium ion concentration. These results are based on the numerical values summarized in table 1.

Discussion. The results suggest that the decrease in both total serum calcium and serum proteins occurring after sleep as well as rest can be accounted for by the increase in plasma volume, i.e., the passage of tissue fluids into the vascular system. This presumption is strengthened by a decrease in the other blood constituents, namely, erythrocytes, hemoglobin and packed red cells after 1½ to 2 hours of rest, and by the increase in the Na and Cl content of the plasma known to occur during sleep. For lymph, and presumably intercellular fluid, contains a greater percentage of Na and Cl than does blood.

Katzenelbogen (9) was of the opinion that if a transfer of calcium did take place, the calcium content of the brain should be greater in the sleeping than in the waking state. However, he found no difference in the whole brain calcium between anesthetized (diallyl-barbituric acid) and control animals. Because small changes in an isolated region may have been masked by the large bulk of brain, he repeated the procedure dividing the brain into various portions and comparing the calcium content of each. Here too, no significant difference between the two groups was observed; although he did find that, in both groups, the calcium content of the hypothalamic region was greater than that of other parts of the brain. He suggested some relation between this and hypothalamic activity. Katzenelbogen's results refute Cloetta's view that the drop in blood calcium observed after sleep is to be explained by a transfer of calcium from the blood to the brain.

This decrease in total calcium can be explained on the basis of dilution by tissue fluids as suggested above. The same mechanism accounts for the fact that ionic calcium remains the same, for tissue fluids contain the same amount of ionic calcium as does blood. The calcium of tissue fluids is in equilibrium with the diffusible calcium of the blood, which McLean and Hastings (11) have shown to be almost entirely in the ionic state. The transfer of fluid explains the absence of a change in ionic calcium in the shorter periods of sleep or rest, but does not explain the increased

calcium ion concentration of the blood after the longer periods of sleep (or rest). It is apparent, however, that the *total amount of calcium* in the blood stream is greater after sleep (or rest) than before, as was previously supposed.

Our results confirm those of Gollwitzer-Meier and Thompson (15) as to hemodilution during rest. This can be considered a result of the decreased blood pressure obtaining during sleep (or rest). A decreased filtration pressure at the arterial end of the capillary bed in the presence of an adequate reabsorption at the venous end by virtue of the serum proteins, would tend to allow fluid to enter the bloodstream, thus increasing the circulating blood volume.

CONCLUSIONS

1. During sleep (5 to 7 hours) both total serum calcium and serum proteins decrease, with a resultant slight but consistent increase in the calcium ion concentration of the serum.
2. During rest without sleep for the same period, similar changes occur.
3. During sleep (and rest) for shorter periods ($1\frac{1}{2}$ to 2 hours) there is a more marked decrease in total serum calcium, but the ionized calcium remains the same.
4. The changes found in the shorter periods of sleep and rest can be correlated with an increase in circulating plasma volume.
5. These results disagree with Cloetta's view that during sleep calcium passes from the blood stream into the tissues.

The writer wishes to express his appreciation to Dr. N. Kleitman and Dr. F. C. McLean for their assistance and friendly criticism in this work. Sincere appreciation is also extended to Miss N. Tupikova, Mr. P. Gold, Mr. M. Davidoff, Mr. M. Bernstein, and others who have so kindly aided in the course of this work.

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ON THE COAGULATION DEFECT IN PEPTONE SHOCK[1]

A Consideration of Antithrombins

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While the existence of anticoagulating substances is generally recognized, relatively little is known of their mode of action and physiological significance. Two common fallacies have moreover added to the complication of the subject: the first being the indiscriminate use of the term, antithrombin, in designating anticlotting agents; the second, the frequent hasty and unwarranted practice of attributing any delayed or defective coagulation of the blood either to a new anticoagulant, or to an increase of the antithrombin normally present in the plasma. In regard to the first, much confusion could be avoided, if one adhered to Howell's criterion of an antithrombin (1), namely, a substance which inhibits the clotting of purified fibrinogen by a solution of thrombin which is relatively free of other constituent of the blood. As to the second, it is difficult to find convincing evidence that clinically any hemorrhagic condition can be attributed to antithrombin, or more broadly, to an anticoagulant. In a recent publication, Quick, Stanley-Brown, and Bancroft (2) presented evidence that in hemophilia the prolonged clotting time appears to be due to a deficiency of available thromboplastin, and that the delayed coagulation observed in certain types of jaundice is probably caused by a marked diminution of prothrombin. In neither type of disease could any abnormal amount of antithrombin be demonstrated.

Fortunately, a condition can however be produced experimentally in which the blood becomes incoagulable as the result of the production of a powerful antithrombin. By injecting peptone intravenously into a dog, a blood is obtained which has lost its power of spontaneous coagulability. Although this condition has been studied repeatedly, its usefulness in obtaining new information on the clotting of blood has by no means been exhausted. In the present investigation the cause of the incoagulability of the blood in peptone shock is restudied, and in the interpretation of the results a correlation of the antithrombins is presented.

[1] This paper was presented before the Forty-eighth meeting of the American Physiological Society at Washington, March, 1936.

EXPERIMENTAL. To produce peptone shock, a well fed unanesthetized dog was injected intravenously with a 20 per cent solution of Witte peptone. The dose was 0.3 gram per kilo of body weight. Blood was taken 5, 30, and 60 minutes after the injection. Usually an oxalated and an unoxalated specimen of blood were collected. For the former, 9 cc. of blood were withdrawn into a syringe containing 1 cc. of 0.1 M sodium oxalate.

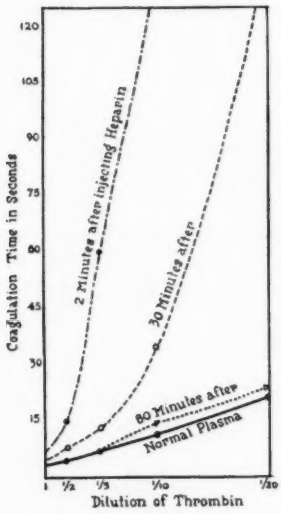

Fig. 1

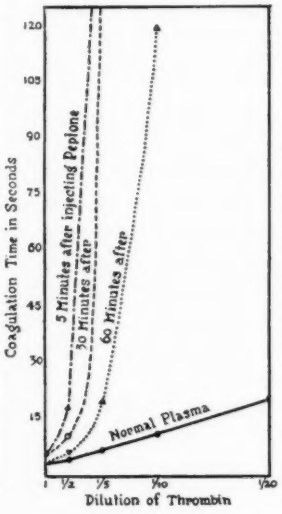

Fig. 2

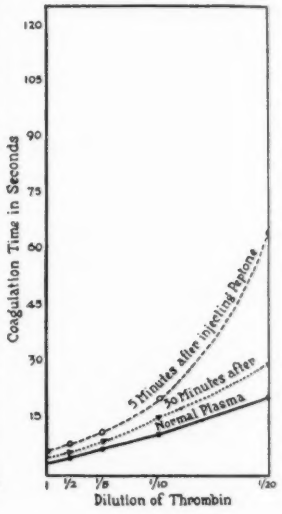

Fig. 3

Fig. 1. Dog 1. Weight 7 kgm. Heparin (70 mgm.) was injected intravenously. No systemic reactions were noted.

Fig. 2. Dog 2. Weight 11.3 kgm. Sixteen cubic centimeters of a 20 per cent solution of Witte peptone were injected intravenously. Moderate systemic reactions promptly occurred: pilomotor effect, defecation, and vomiting.

Fig. 3. Dog 3. Weight 8.5 kgm. Twelve cubic centimeters of a 20 per cent solution of Witte peptone were injected intravenously. No systemic reactions were observed.

Heparin. A preparation made from beef lung according to the directions of Charles and Scott (3) was used. It had about the same potency as the commercial product of Hynson, Westcott, and Dunning.

Thrombin. This agent was prepared as in the preceding study (4) from human plasma following the method developed by Eagle (5).

Antithrombin titration. A series of dilutions of thrombin was prepared by mixing the concentrated preparation of thrombin with appropriate amounts of distilled water; 0.2 cc. of plasma was placed in a small tube

(13 by 100 mm.), quickly mixed with 0.1 cc. of diluted thrombin, and the clotting time recorded. All determinations were made in a water bath maintained at 37 to 39°C.

The effect of injected heparin on the clotting time of plasma by known concentrations of thrombin is shown in figure 1. Only three experiments on the intravenous injection of peptone were selected for the present presentation since these illustrate adequately the usual variations in antithrombic action obtained in peptone shock. Figures 2 and 3, and table 1.

The curves obtained by plotting the clotting time of plasma containing small amounts of heparin against progressive dilutions of thrombin are remarkably similar to those found by plotting the clotting time of peptone plasma against the same dilutions of thrombin. The inhibition of clotting resulting either from the injection of heparin or from peptone shock is of short duration. Often waning of the effect can be noted in

TABLE 1

The production of antithrombin by the injection of peptone

	CLOTTING TIME IN SECOND			
Concentration of thrombin	1	1/2	1/5	1/10
Oxalated plasma before peptone injection	3.5	5	7	11
Oxalated plasma 20 minutes after peptone injection	8	24	No clot	
Unoxalated plasma 20 minutes after peptone injection	9	29	No clot	

Dog 4. Weight 7 kgm. Ten cubic centimeters of 20 per cent Witte peptone were injected intravenously. Mild systemic reactions were noted.

30 minutes, and usually normal coagulation is restored during the second hour. The amount even of impure heparin required to produce the same degree of antithrombin activity as is generally found in the plasma after the injection of peptone is small. It is not surprising therefore that its isolation with the present available methods has been unsuccessful. Nevertheless, it seems a fairly reasonable assumption that the blood in peptone shock contains a heparin-like substance, although final proof requires the actual isolation and identification of this anticoagulant. Interestingly, the response to the injection of peptone varies rather greatly. Sometimes the reaction is so slight that the animal exhibits no systemic effects and the blood shows no distinct aberration from the normal in respect to clotting. Nevertheless, on determining the clotting time of such a plasma with progressive dilutions of thrombin, the presence of an anticoagulant can readily be detected as shown in figure 3.

While it appears reasonably certain that a heparin-like substance is the cause of the incoagulability of peptone plasma, the perplexing problem of the origin and source of this anticlotting agent remains unsolved.

Heparin can be prepared from many different types of body tissues, but curiously, this substance cannot be demonstrated in fresh tissue, not even in the lungs, which constitute one of its richest sources (6). To obtain heparin from any tissue, strong reagents and relatively vigorous methods must be employed. In view of this it is probably more correct to state that a certain organ or tissue will, on subjecting it to a series of chemical reactions, yield a certain amount of heparin, rather than saying that it contains this amount, implying thereby that this quantity is readily available. Evidence to show that the organism can produce heparin by breaking down body tissues is still lacking. While some investigators have concluded that the liver is responsible for the changes in coagulation produced by the injection of peptone, others have obtained experimental results which are contrary to this assumption. This subject is fully discussed by Wöhlisch (7) in his comprehensive review. Hiruma (8) claimed that he was able to prepare an antithrombin from the intima of the aorta taken from a freshly killed dog or rabbit. This finding suggests that perhaps the reticulo-endothelial system might be the source of the antithrombin. It has not been possible however to confirm the observations of Hiruma although his directions were followed explicitly. There is also the possibility that peptone itself may be the source of heparin, since Brüda (9) has reported that an anticoagulant resembling heparin can be prepared from Witte peptone. Mills (10) has proposed the ingenious theory that in peptone shock there is an overproduction of cephalin-combining proteins which rob thrombin of cephalin and thus reconvert it to inactive prothrombin. It is very probable that Mills' explanation as well as his theory of the coagulation of the blood are based on rather uncertain assumptions. The writer (11) has reported experimental results which strongly indicate that thromboplastin and not cephalin brings about the activation of prothrombin; and furthermore that the thrombin which disappears from the serum is not regenerated by an extract of brain, which incidentally contains both thromboplastin and cephalin. The latter finding confirms the earlier work of Gasser (12). The results presented in this paper support Howell's (13) view that the coagulability of peptone plasma is due to a substance similar or identical with heparin, but no solution as to the origin of this substance can be offered.

It is a well known fact that plasma obtained after the injection of peptone as well as plasma containing heparin can be made to clot by the addition of thromboplastin. This finding has led to the assumption that thromboplastin neutralizes heparin. The writer (4) has recently reported findings which have led him to conclude that thromboplastin does not directly neutralize heparin. Another explanation for the effectiveness of thromboplastin in bringing about clotting of a blood made incoagulable with peptone must be sought. The amount of heparin in peptone plasma pro-

duces sufficient antithrombin presumably to neutralize all the thrombin that is formed and thus prevents coagulation. The amount of thrombin even in normal blood is limited by the available thromboplastin. The coagulation time of human blood as measured by the method of Lee and White is from 5 to 8 minutes, but on adding a minute amount of thromboplastin it can be made to clot in less than 30 seconds, indicating that the amount of prothrombin far exceeds that of thromboplastin. The limited amount of thromboplastin can be attributed in part to the slowness with which platelets disintegrate, and according to Zucker (14) heparin even increases the resistance of platelets to lysis. Obviously, when thromboplastin is added to plasma containing heparin, additional thrombin is formed and when this exceeds the amount which can be neutralized by the antithrombin, clotting ensues. It is to be expected that any means that hasten the disintegration of platelets may, if sufficiently effective, bring about clotting, and this perhaps explains why a simple procedure such as the dilution of the plasma may cause coagulation.

In view of the fact that experimental evidence indites an antithrombin as the basic cause of the defective clotting in peptone shock, it follows that a comprehensive understanding of the coagulation disturbance in this condition is contingent on a more satisfactory solution of the problem of the antithrombins. To this end a number of puzzling experimental observations must be correlated. It will be recalled that one obtains a straight line by plotting the clotting time of plasma or purified fibrinogen against increasing dilutions of thrombin, and furthermore that the addition of heparin to plasma destroys this direct linear relationship. The direct ratio between the clotting time of plasma and the concentration of thrombin demonstrates that plasma does not contain an antithrombin which interferes with coagulation. When heparin is added to blood, a powerful antithrombin is formed which promptly neutralizes the action of thrombin. As long however as the concentration of the thrombin exceeds that of the antithrombin, coagulation is only delayed, but when the latter equals or exceeds the quantity of thrombin, coagulation is completely inhibited. This is illustrated plainly in figure 1. To avoid confusion it is probably best to refer hereafter to this anticoagulant as heparin-antithrombin.

The statement that the direct ratio found between the clotting time of plasma and the concentration of thrombin indicates the absence of antithrombin in normal blood seems a priore paradoxical. It is well known that the potency of thrombin is rapidly lost in serum or even in plasma from which the fibrinogen has been previously removed. The fact that thrombin can be regenerated by successively acidifying and neutralizing the serum, shows that a neutralization and not a destruction of thrombin has occurred. Blood must therefore contain a substance which can neutralize thrombin, in other words, an antithrombin. The quantity of this

antithrombin must moreover be large since serum can inactivate not only all the thrombin formed from the prothrombin originally present in the blood but additional amounts as well. But this normal antithrombin is obviously quite different from the heparin-antithrombin. In the first place, it does not influence the linear relationship existing between the clotting time of plasma and the concentration of thrombin, which heparin promptly destroys; and in the second place, it inactivates thrombin relatively slowly in contrast to the rapid, almost instantaneous neutralizing action of the heparin-antithrombin. The normal and slowly acting antithrombin is present both in serum and plasma. In a previous paper (4), the writer made the error of stating that plasma does not contain antithrombin. Gasser (12) has demonstrated however that plasma from which the fibrinogen has been removed can neutralize thrombin, and the writer likewise has found that plasma has essentially the same power to inactivate thrombin as has serum.

To correlate the experimental observations made on normal antithrombin and on heparin-antithrombin and to account for their differences in behavior, the following hypothesis is offered. When thrombin is formed, it immediately combines with its normal substrate, fibrinogen, which it converts to fibrin, and thereby is reliberated becoming thus available to produce further coagulation. The successive crops of fibrin observed when a weak solution of thrombin is employed can be accounted for by assuming such a union of thrombin with fibrinogen and a subsequent reliberation. When all of the fibrinogen has been coagulated, another constituent of the serum, most probably one or more of the blood proteins combine with thrombin, and this substance or group of substances constitutes the normal antithrombin of the serum or plasma. Since the affinity of the latter for thrombin is relatively weak, it cannot compete with fibrinogen for this agent; therefore it does not interfere with normal coagulation. For the same reason the union of the normal antithrombin of the blood occurs relatively slowly with thrombin and this accounts for the gradual disappearance of thrombin from serum. When the union has been completed, however, only strenuous procedures such as acidifying the serum with subsequent neutralization can effect a partial regeneration of thrombin. Since the amount of normal antithrombin has no demonstrable influence on coagulation, and since it far exceeds even normally the quantity necessary to neutralize all of the potentially available thrombin of the blood, it seems fairly certain that even wide variations in its concentration are of little or no practical significance. One can therefore look upon the normal antithrombin of the blood as serving the useful purpose of acting figuratively as a sponge to absorb the excess of thrombin after coagulation has been completed.

When heparin is added to blood, it gives rise to a strong antithrombin.

One can postulate that heparin combines with a constituent of the blood, probably a serum protein, thereby producing a substance which has an affinity for thrombin which is even greater than that of fibrinogen; consequently thrombin is bound quantitatively and is inactivated by this heparin antithrombin before it can react with fibrinogen, thus preventing the first essential step in coagulation. By assuming that the heparin-antithrombin is a protein-heparin compound, one can easily explain the puzzling fact that heparin which is thermostable forms a thermolabile antithrombin.

Interestingly, other substances have been found which behave like heparin in that they produce a new and potent antithrombin in plasma. The writer has found that the dye, Calcomine fast pink 2Bl[2], is such an anticoagulant. This substance is structurally similar to the compounds studied by Huggett and Rowe (15). Germanin[2] (Bayer 205) likewise was found to produce an antithrombin in plasma. Jorpes (16) has recently called attention to the high content of sulfates in the ash of heparin. Incidentally, Howell made this observation in his original studies of this agent. Jorpes postulates that heparin is a chondroitinpolysulphonic acid, and points out the interesting fact that other anticoagulants such as Germanin, Liquoid Roche, and various dyes, contain sulphonic acid groups. From these considerations one is inclined to believe that heparin may have a relatively simple structure.

SUMMARY

By plotting the clotting times of dog plasma obtained after the intravenous injection of peptone against progressive dilutions of thrombin, a curve is obtained which is very similar to that found by plotting the clotting times of plasma containing heparin against the same dilutions of thrombin. This finding strongly suggests that the incoagulability of the blood in peptone shock is due to the production of an anticoagulant similar to or identical with heparin.

The antithrombin produced by the injection of peptone or by adding heparin to plasma differs markedly from the antithrombin normally occurring in the blood. The latter apparently does not interfere with normal coagulation and only slowly neutralizes thrombin, whereas the heparin-antithrombin even in minute amounts delays or completely inhibits coagulation by promptly neutralizing the thrombin before it can act on fibrinogen.

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[2] The dye, Calcomine fast pink 2Bl was kindly furnished by the Calco Chemical Company, and the Germanin by the Winthrop Chemical Company.

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THE LIPID METABOLISM OF THE HYPOPHYSECTOMIZED DOG AND THE LIPID AND CARBOHYDRATE METABOLISM OF THE HYPOPHYSECTOMIZED-DEPANCREATIZED DOG[1]

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Although the carbohydrate metabolism of the hypophysectomized dog has been extensively studied, little information is available regarding the relation of the pituitary gland to lipid metabolism. The obesity that has at times been observed to follow hypophyseal interferences cannot be ascribed at present to damage of the gland itself, for it is now common knowledge that rats that have survived the discrete removal of the gland for long periods of time show no excessive depositions of fat (1). Three findings, however, suggest that the gland may influence lipid metabolism: 1, the isolation from the anterior pituitary gland of a factor capable of increasing acetonuria in rats (2, 3); 2, the reduction of the acetonuria of the depancreatized dog by means of hypophysectomy (4); 3, the fall in blood cholesterol produced by chronic injections of thyreotropic hormone (5). In regard to the first two findings it should be noted, however, that the relative rôles of fat and protein in the acetone body changes still remain to be determined.

In the present investigation, a detailed study has been made of the blood and liver lipids of the completely hypophysectomized dog. Despite the fact that alleviation of diabetic signs and prolongation of life for several months have been recorded by Houssay (6) and others in the case of dogs in which both hypophysis and pancreas are excised, little is known about the lipid changes in the livers of such animals, although it is becoming apparent that an increased lipid content in the liver is a significant finding in the depancreatized dog receiving no insulin. In the present study, therefore, the lipid content of the liver in relation to the carbohydrate changes in blood, urine and liver has been determined in the hypophysectomized-depancreatized dog.

EXPERIMENTAL. The intracranial manipulations to which the hypo-

[1] The expense of this investigation was defrayed in part by a grant to one of us (I.L.C.) from the Research Board of the University of California, Berkeley.

physectomized dogs were subjected have been described elsewhere (7). Two groups of "operated-control dogs" were provided. In the first (3 dogs) craniotomy and retraction of the right temporal lobe were performed, whereas in the second (3 dogs) the attachment between hypophysis and sella turcica was severed after the right temporal lobe had been retracted.

TABLE 1

Whole blood lipids (postabsorptive) of completely hypophysectomized dogs†*

DOG	INTERVAL SINCE HYPOPHYSECTOMY	CHOLESTEROL				TOTAL LIPID	TOTAL FATTY ACIDS	PHOSPHOLIPID	RESIDUAL FATTY ACIDS‡
		Total	Free	Ester					
	weeks	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent of total*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
HI	20	188	135	53	28	613	425	380	131
HII	30	149	121	28	19	601	452	353	195
HIII	31	222	135	87	39	703	481	406	145
HIV	25	269	165	104	39	872	603	516	180
HV	25	225	133	92	41	653	428	395	96
HVI	24	159	123	36	23	507	348		
HVII	23	170	134	36	21	571	401	362	132
H8	5	162	119	43	27	534	372		
H8	6	164	117	47	29	554	390		
H9	9	166	122	44	26	549	383	350	116
H10	9	181	139	42	23	605	424	376	142
H15	16	196	123	73	37	631	435	407	109
H16	12	189	121	68	36	592	403	382	97
H17	12	177	110	67	38	572	395	353	109
H18	10	202	119	83	41	592	390	346	97
H19	13	174	117	57	33	562	388	359	106
HA	28	144	114	30	21	468	324	291	107
Maximum.....		269	165	104	41	872	603	516	195
Minimum.....		144	110	28	19	468	324	291	96
Mean.....		184	126	58	31	599	414	377	126

* The animals were fed twice daily at 8:00 a.m. and at 4:00 p.m., while blood for analysis was taken just before the morning meal.

† Completeness of hypophysectomy checked by serial sections of whole hypophyseal region at necropsy.

‡ Derived chiefly from neutral fat.

Whole blood was used for lipid analyses; the oxidative methods employed have been previously recorded (8). The methods for sampling the liver for lipid and glycogen determinations, as well as the analytical procedures employed have been recorded elsewhere (9, 10). The copper-iodometric reagent of Shaffer and Somogyi (11) was used for sugar determinations in urine. Acetone bodies in urine were determined by the Van Slyke method (12) and nitrogen by that of Kjeldahl.

1. *Blood lipids of hypophysectomized and operated control dogs.* The values obtained for 16 hypophysectomized dogs are shown in table 1, while the maximum, minimum and mean values found in 41 normal and 6 control dogs are contained in table 2.

2. *Liver lipids of completely hypophysectomized dogs.* The lipids found in the postabsorptive state in the livers of normal and hypophysectomized dogs maintained under the same nutritional conditions are shown in table 3.

3. *Carbohydrate and lipid metabolism of the completely hypophysectomized-depancreatized dogs.* The protocols of 3 dogs subjected to excision of all hypophyseal and pancreatic tissues are given below.

TABLE 2

Maximum, minimum and mean values for whole blood lipids of operated-control and normal dogs

(Values expressed in milligrams per 100 cc. unless otherwise stated)

	OPERATED CONTROL*			NORMAL†		
	Maximum	Minimum	Mean	Maximum	Minimum	Mean
Cholesterol:						
Total	192	134	169	205	122	156
Free	152	108	131	144	94	119
Esterified	53	26	38	66	22	36
Ester (per cent of total)	28	18	22	34	18	23
Total fatty acids	403	340	372	440	281	362
Phospholipid	425	298	364	405	248	324
Residual fatty acids	150	50	96	203	62	114
Total lipid	595	482	549	644	403	519

* 6 dogs.
† 41 dogs.

HV was hypophysectomized on December 27, 1933, and depancreatized on July 2, 1934, at which time the dog weighed 11.1 kilos. It was injected with small doses of insulin after pancreatectomy and at the end of 2 weeks was receiving twice daily, at 9:00 a.m. and at 4:00 p.m., 8 units of insulin and a diet mixture consisting of 225 grams of lean meat, 60 grams of sucrose, 5 grams of bone ash, and vitamin supplements. On September 11, at 9:00 a.m., the animal received its last injection of insulin. It died on September 29. Glucose was present in the urine during the whole period of survival. Eighty-four and 57 grams were excreted during the first and second days respectively following withdrawal of insulin injections, while during the following period the amount excreted varied from 2 to 18 grams per 24 hours. On the 9th day of observation the blood sugar was 313 mgm. per cent, and the blood sample obtained just before death contained

433 mgm. per cent. With the exception of the first 2 days, acetone bodies were present in the urine. The liver was removed immediately after the animal died; its lipid content is shown in table 3.

Dog HIX was hypophysectomized on October 8, 1934, and was depancreatized on January 10, 1935. The injection of insulin was begun on the third day after pancreatectomy, and by February 11 this animal was receiving 3 units in the morning and 4 units in the evening. On that day the postabsorptive blood sugar taken before the administration of the

TABLE 3

Liver lipids of normal, hypophysectomized and hypophysectomized-depancreatized dogs

	DOG	WEIGHT	INTERVAL SINCE HYPOPHYSECTOMY	CHOLESTEROL				TOTAL LIPID	TOTAL FATTY ACIDS	PHOSPHOLIPID	RESIDUAL FATTY ACIDS
				Total	Free	Ester					
		kgm.	*months*	*per cent*	*per cent*	*per cent*	*per cent of total cholesterol*	*per cent*	*per cent*	*per cent*	*per cent*
Normal*	N24	8.5		0.27	0.27	0.00	0	4.05	2.61	2.42	0.99
	N25	9.1		0.25	0.22	0.03	12	4.19	2.06	2.17	0.59
	N26	7.8		0.24	0.23	0.01	4	3.96	2.69	2.41	1.06
	N27	6.2		0.23	0.22	0.01	4	3.02	2.01	1.92	0.71
Hypophysectomized*	H15	14.0	4	0.23	0.19	0.04	17	3.58	2.34	1.99	0.98
	H16	13.7	3	0.23	0.22	0.01	4	3.58	2.30	2.04	0.92
	H17	8.2	3	0.27	0.24	0.03	11	3.74	2.67	1.95	1.34
	H18	15.8	2.5	0.24	0.21	0.03	13	3.78	2.70	1.94	1.37
	H19	9.0	4	0.23	0.18	0.05	22	3.84	2.60	1.87	1.32
Hypophysectomized-depancreatized	HII	9.0	7.5†	0.25	0.16	0.09	36	26.4	23.9	1.49	22.8
	HV	8.6	9‡	0.30	0.10	0.20	67	66.7	61.7		
	HIX	5.8	8§	0.24	0.09	0.15	63	43.2	38.5	1.70	37.2

* Obtained when dogs were in postabsorptive state.

† Deprived of insulin supply for 4 days.

‡ Deprived of insulin supply for 18 days.

§ Deprived of insulin supply for 3.5 months.

morning insulin was 305 mgm. per cent, and the animal's weight was 10.3 kilos. It was fed meat, sucrose, a fish and cereal mixture, milk and raw pancreas. On February 16 the fasting blood sugar was 318 mgm. per cent and the weight 10.7 kilos. This dog received its last injection of insulin on February 19. Its subsequent history is recorded in table 4. Although blood and urine were analyzed daily during its 3.5 months of survival after the discontinuation of insulin, the results of only 12 typical days are recorded. On June 4, when the animal was in a weakened and

emaciated condition, it was injected with sodium amytal and the liver removed for glycogen and lipid determinations. *The glycogen content of the liver was 2.35 per cent,* while the lipid content is shown in table 3.

Dog HII was hypophysectomized on November 10, 1933. Pancreatectomy was performed on June 25, 1934, and the liver removed for lipid analysis on June 29. Following pancreatectomy the animal received neither food nor insulin. The lipid content of the liver is shown in table 3.

At necropsy no pancreatic tissue was found in these 3 dogs. Serial sections were also made of the whole hypophyseal region. There was a

TABLE 4

Blood sugar changes and the excretion of sugar, nitrogen and acetone bodies by hypophysectomized-depancreatized dog HIX

DATE	WEIGHT	URINE						BLOOD SUGAR	FOOD INGESTED*
		Volume	Hours collected	Glucose	Nitrogen	Acetoacetic acid	β-Hydroxybutyric acid		
	kgm.	*cc.*		*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm. per cent*	
Feb. 21	10.0	370	24	15.3	9.1	0	0	243	292 gm. meat
Feb. 27	9.5	280	24	19.5	8.3	13	67	266	125 gm. meat
Mar. 3	9.2	370	24	17.1	13.2	45	120	236	195 gm. meat
Mar. 16	8.5	160	24	8.8	4.4	11	94	270	150 gm. meat
Mar. 27	8.2	280	48	10.4	7.4	19	138	199	260 gm. meat
Apr. 14	7.4	250	24	11.3	5.3	35	209	204	130 gm. meat
Apr. 27	6.9	150	48	4.3	5.5	23	185	241	372 gm. meat
May 8	6.5	145	24	6.7	4.3	14	146	258	288 gm. meat
May 18	6.2	260	24	10.5	6.7	20	150	292	268 gm. meat
June 2	6.0	250	24	6.6	5.9	17	96	260	245 gm. meat
June 3	5.8	245	24	7.9	5.2	5	63		439 gm. meat
June 4	5.8	390	24	12.4	8.7	19	147	259	

* Vitamin B was supplied as a concentrate of rice bran, and A and D as cod liver oil.

complete absence of anterior, posterior, and intermediate lobes. The thyroid glands were atrophied and contained flattened epithelium.

Discussion. The results of the present investigation show that, despite the complete absence of hypophyseal tissue for as long as 4 months, the liver of the dog is strikingly normal in its lipid constituents, namely, cholesterol, both free and esterified, phospholipids and fatty acids. A study of the lipid content of the blood, however, yielded variable results. While values for various lipid constituents were found above the highest normal in 5 of the dogs examined several months after excision of the hypophysis, nevertheless the level of all lipid constituents in the rest of the 16 hypophysectomized dogs studied was essentially normal. In contrast to the

conclusions of Muñoz (13), it should therefore be noted that changes in the lipid concentration of the blood, when they did occur, were always in the direction of a rise. This was particularly marked in the case of cholesterol esters. No striking departure from normal was found in the blood content of the various lipid constituents in the 6 control dogs, despite the fact that the degree of cerebral manipulation in these animals varied from retraction of the temporal lobe to liberation of the pituitary gland from its attachment to the sella turcica.

A gain in weight may occur in dogs following hypophysectomy (7), and the question therefore arises whether the increased lipid content of the blood observed in a few of these animals was the result of the absence of hypophyseal tissue or was secondary to the change in their nutritional state. Although the gain in weight cannot entirely be ruled out as a factor associated with these high lipid values, nevertheless it should be noted that normal blood lipids were found in a large number of hypophysectomized and operated-control dogs in which considerable gains in weight followed the intracranial operation.

Further evidence that the lipid metabolism of the liver is not influenced by the absence of pituitary hormones was found in the hypophysectomized-depancreatized dogs. Previous excision of all hypophyseal tissue failed to inhibit the deposition of lipids that follows removal of the pancreas. Thus HV showed a fatty acid content of 62 per cent in its liver, while 4 days after pancreatectomy HII accumulated 24 per cent.

From the results obtained in the present investigation, it is clear that insulin is not only beneficial but also essential in the survival of the completely hypophysectomized-depancreatized dog. Although after removal of both glands HV was maintained by means of insulin in good nutritional state and with no impairment of appetite for well over 2 months, it lived for only 2 weeks after cessation of insulin administration, during which time it not only showed a marked loss of appetite and weight, but also excreted glucose and acetone bodies. The failure of HIX following cessation of insulin administration was not so marked as in HV and its period of survival (3.5 months) is more in keeping with previous observations of Houssay (6) and others. During this time, however, it showed all manifestations of diabetes, including acetonuria. The variations in the degrees of diabetes manifested by hypophysectomized-depancreatized dogs are undoubtedly related in part to differences in nutritional states of the animals. After both hypophysectomy and pancreatectomy HV was in an exceptionally well nourished state and possessed a vigorous appetite. Although hypersensitive to insulin after hypophysectomy, this animal tolerated well 16 units of insulin daily after the second operation. HIX, on the other hand, showed a poor appetite after pancreatectomy, and, though blood sugars above 300 mgm. were observed, it displayed marked

sensitivity to insulin during the period immediately following this operation. Since the significance of the nutritional state of the animal in determining the degree and course of diabetes is not always realized, it should be noted here that as early as 1921 Joslin found severe hypoglycemia in diabetic patients who had been subjected to Allen's starvation treatment (14).

Although the liver of the diabetic dog has not entirely lost the capacity to store glycogen, it is now well known that, shortly after loss of insulin supply by pancreatectomy, very small amounts of glycogen are left in the liver (15, 16). This, however, is not the case with the hypophysectomized-depancreatized dog. Despite the fact that HIX had been deprived of known sources of insulin for 3.5 months, its liver contained well over 2 per cent glycogen. Collip found 1.6 and 1.8 per cent glycogen in the livers of 2 hypophysectomized-depancreatized dogs (17). The removal of hypophyseal tissues thus facilitates the storage of glycogen in the diabetic dog's liver, an observation that is in keeping with a previous finding that in the absence of hypophyseal hormones there is no interference in the capacity of the dog to store glycogen (10).

SUMMARY

1. Although the lipid content of the blood obtained from some of the dogs after complete excision of the hypophysis was significantly above normal, all lipid constituents, namely, total fatty acids, phospholipid and free and esterified cholesterol, were present in normal amounts in 11 of the 16 hypophysectomized dogs studied.

2. In the absence of all hypophyseal tissue, the various lipid constituents, namely, total fatty acids, phospholipid and free and esterified cholesterol, are contained in normal amounts in the liver.

3. The complete excision of the hypophysis does not inhibit the rapid accumulation of large amounts of lipids in the liver following pancreatectomy.

4. The carbohydrate changes in the hypophysectomized-depancreatized dog and its capacity to store glycogen in the liver are recorded and discussed.

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CALCULATION OF CARDIAC OUTPUT FROM BLOOD PRESSURE MEASUREMENTS BEFORE AND AFTER MEALS

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The calculation of cardiac output and of the effective peripheral resistance from measurements of blood pressure and pulse wave velocity under basal conditions in man has already been described (1935), and it was pointed out at that time that the equations were mainly empirical, and that their validity for other conditions would have to be established. The effects of meals on the circulation provide a useful test, since during absorption of food there is undoubtedly a vasodilatation in the splanchnic area, and also an increased blood flow in the limbs according to the work of Burton and Murlin (1935), and of Herrick, Essex, Mann and Baldes (1934). Consequently, 11 experiments have been made on 4 subjects, 3 of whom were utilised in the previous study (subjects 4, 8 and 10). The additional subject (14) was a girl 22 years of age with a surface area of 1.66 square meters. In these experiments estimations of cardiac output in the basal condition have first been made, both by calculation and by the acetylene method. Estimates of cardiac output by both methods, 40 to 140 minutes after the middle of a heavy meal, have alternated with one another in varying order. However, in experiment 1, estimates on cardiac output were made only from blood pressure and pulse wave velocity measurements, and in experiment 2 on the same subject, the observations were similarly limited to the acetylene method.

METHODS. The methods used were, for the most part, the same as those previously described. In the acetylene estimations determinations of the arterio-venous difference were made occasionally from two samples obtained after 13 to 14 and 18 to 20 seconds of the rebreathing procedure; usually at least 3 samples were taken at approximately 14, 19 and 23 seconds, and sometimes a fourth sample at 30 seconds was added. While the later samples were used as a check, calculations were always made from the first pair of samples, where their validity was established. In some of the experiments the initial mixture contained high percentages of oxygen, and of carbon dioxide to test the accuracy of the acetylene procedure, and these samples were analysed in a special analyser developed for the purpose. This analyser, the variations

observed in late samples, and the effect of the original gas mixture upon them will be described later.

Technic of preservation of acetylene samples. In some experiments it was necessary to take 16 gas samples for analysis, and it was found that samples kept over mercury under pressure for 24 hours or even less often showed a significant loss of acetylene. Such loss was not due to leak, nor to absorption of acetylene by grease. It was accompanied by an increase in CO_2 and a decrease in O_2, but these changes were not proportional to the acetylene loss, and the main change was an increase in the unabsorbable gas called nitrogen. Control experiments showed that this loss was not appreciable if the mercury and tubes were absolutely clean. In the presence of oxides or other mercury compounds, in the mercury or on the glass, acetylene disappeared. The changes observed when a sample was kept 24 hours over clean mercury in a dirty bottle are shown in table 1. The changes shown are not maximal; also significant changes may develop in a much shorter time, certainly within 8 hours. Such losses may be avoided if the tubes are evacuated with a Hyvac pump, and are tested with the spark of a Tesla coil. Mercury then only comes in contact with the gas at the time of analysis.

TABLE 1

	CLEAN BLOTTLE AND MERCURY		DIRTY BOTTLE AND CLEAN MERCURY	
	1st day	2nd day	1st day	2nd day
CO_2	4.85	4.87	4.89	4.98
C_2H_2	11.96	11.94	11.94	11.67
O_2	13.65	13.66	13.68	13.66
N_2	69.54	69.53	69.49	69.69

The blood pressure procedure was that previously described. In estimating pulse wave velocity the procedure was also the same, except that apex beats were not employed. Instead, the subject lay upon his back and a heavy metal bar was fixed to rods attached to the sides of the bed, so that it crossed above the subject's chest at about the level of the junction of the 3rd rib with the sternum. To this bar was attached a glycerine capsule tambour, which was arranged to press firmly on the center of the sternum somewhere between the attachments of the 2nd and 4th ribs. This tambour was connected to an optically recording segment capsule, and a side tube from the connecting tubing was adjusted with a clamp so as to be always slightly open, to damp out the effects of respiratory movements. Such a system has always given similar curves on the same subject, not only under basal conditions, but also after meals or with the subject in a hot or neutral bath. The great variability, that is so characteristic of apex beat records, is no longer seen. The method has been applied to three other subjects besides those used in these experiments. In all cases it has been checked by the use of simultaneous electrocardio-

grams, and in addition in some cases by simultaneous records of the apex beat, or of lateral movements of the chest wall. In 6 of the 7 subjects used the curves obtained have a general similarity with one another; in the oldest subject (10 of the previous series), with a somewhat bizarre electrocardiogram and a long P R interval, the curve appears to be of a different type, and not analysable on the same principles; the curves are, however, always reproducible for that subject.

The subject's head and shoulders were throughout somewhat raised on pillows; during the rebreathing procedure in acetylene estimations this posture was exaggerated to a semi-reclining position to facilitate mixing in the lungs, but the subject was not allowed to adjust his position by his own efforts.

Of the 11 experiments, 8 were performed in the morning with the subjects without breakfast and after a rest of $\frac{3}{4}$ hour to 2 hours. The other three experiments started with the subject approximately basal, in the afternoon some 5 to 7 hours after breakfast. In three of the meals alcohol was taken; in all coffee was drunk, and the meal was made as heavy as possible. The subjects sat up to take the meal. The reaction appeared to vary somewhat in magnitude and character, but no relation of such variations to the individual subject, to the character or time of the meal, or to the season of the year could be detected.

EXPERIMENTAL MATERIAL. *Interpretation of the sternal pulsations.* Typical records of sternal pulsations are shown in figures 1 to 3, where the relation of the pulsations to electrocardiograms may be seen. Slow waves are present during auricular contraction. At about the peak of the R wave, or 0.01 second earlier, the start of ventricular contraction, *1*, appears to be indicated by a slight dip or plateau followed by a steep rise; 0.035 second to 0.06 second later the rise ends abruptly in a plateau, *2*, (figs. 1 and 3) or more commonly in a brisk downstroke (fig. 2). This abrupt change, occurring usually at the end of the S wave of the electrocardiogram, is too late to be associated with the start of ventricular contraction, and from its time relations is probably dependent on either right or left ventricular ejection. At the end of ventricular contraction the 2nd heart sound (2nd) can usually be seen on the records. A record obtained in subject 10 is shown in figure 4. Here any interpretation of the rapid downstroke, marked 1 as an indiction of ventricular ejection appears impossible, in spite of the superficial resemblance to the other curves, for this rapid downstroke is often contemporaneous with the peak of the R wave. The rapid downstroke is in this subject broken either by a sudden or a gradual slowing (marked *2* in fig. 4), and this relative upstroke is usually associated with secondary vibrations in the subclavian pulse of the type that accompany the downstrokes in the other subjects (see fig. 2). This point, *2*, also gives values for the pulse wave velocity to the subclavian of the same order as those derived from apex beats.

The sternal records were originally developed with the intention of measuring recoil of the type described by Y. Henderson in 1905. Records were first obtained from a bed suspended from the ceiling, but it was found that equally good records of recoil could be obtained optically from a glycerine tambour pressed on to the top of the head with the subject lying on an ordinary bed; such records showed the recoil

movements of the subject relative to the bed. Since the recoil effects so demonstrated showed lag and could not be readily used for timing, the more direct effects of the heart on the thoracic wall were recorded. That recoil movements affect such records, whether obtained from the head or the chest, is certain, but such recoil effects may be of peripheral origin. For instance, if a subject lay on one side on the swinging bed with the hips flexed, the bed was moved by the recoil of blood passing round the angle, and the femoral pulsation could be as readily recorded from the lateral movement of the bed opposite the buttocks, as directly from the femoral artery in the groin. In sternal records attempts have been made to distinguish the recoil effects from those due to the movement of one part of the chest relative to another as described by Dressler (1933), and to determine the cause of the differences

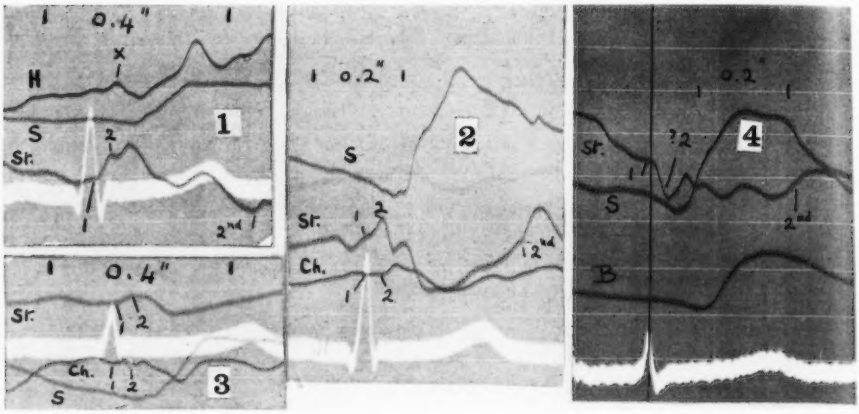

Fig. 1. Subject 8. Basal. Head, subclavian, sternal pulsations and ECG lead II from above downwards. For description see text.

Fig. 2. Subject 8. Basal. Subclavian, sternal, chest pulsations.

Fig. 3. Subject 14. Basal. Marks as in figure 2.

Fig. 4. Subject 10. Post-prandial. Sternal, subclavian and brachial pulsations. The vertical line drawn follows the peak of R by an interval equivalent to that of the transmission time of the recording system for the other records.

observed in subject 10. The relation of the sternal pulsations to the head pulsations, and to lateral movements of the chest wall may be briefly described.

In figure 1 simultaneous records are shown of the sternal pulsation and that of the movements of the top of the head relative to the bed. It will be noted that the head pulsations are of the type recorded by Yandell Henderson (loc. cit.). However, it does not appear possible to interpret the foot-ward movement of the body following the point marked *X*, as due to recoil from the heart ejection, and the later headward movement to the recoil from the passage of the blood round the arch of the aorta, unless these movements develop slowly and lag behind the start of recoil. Not only do the changes occur too late relative to the electrocardiogram, but also the subclavian pulse anticipates the time indicated for the wave to reach the aortic arch on such interpretations. The head movements are not suitable for timing. The start of ejection probably occurs during the earlier upstroke (head-ward movement) of

the head pulsations preceding point *X*; it is also associated with an outward movement of the ribs in the mid-axillary line on the *right* side (fig. 2 and 3). In subject 10 this outward movement of the ribs is associated with the upstroke of the sternal record, which is assumed to indicate ejection. Lateral movements of the left chest wall vary with the apex beat and are not constant.

On these grounds the points indicated *1* and *2* in figures 1 to 4 have been taken as contemporaneous with the start of ventricular contraction and ejection, and pulse wave velocities have been calculated on this basis. The sternal pulsation, owing to its constancy in any one subject, is a more satisfactory indicator than is the apex beat, but no data have been obtained on subjects in whom a marked asynchronism of the right and left ventricles was indicated, and at present there is no proof whether the criteria used are of left or right ventricular origin. The time relations of the sternal pulsations relative to the electrical changes are indicated in table 2.

TABLE 2

Time relations of sternal pulsation relative to E. C. G. (Averages)

SUBJECT	BASAL				AFTER MEAL			
	Q to sternal 1	Q to sternal 2	Peak of R to sternal 2	T to 2nd sound	Q to sternal 1	Q to sternal 2	Peak of R to sternal 2	T to 2nd sound
14	0.030	0.077	0.047	0.016	0.028	0.070	0.034	−0.002
4	0.030	0.066	0.026	0.035	0.034	0.067	0.027	+0.010
8	0.042	0.084	0.042	0.012	0.041	0.079	0.035	−0.007
10	0.020	0.061	0.023	0.006	0.017	0.052	0.012	−0.031
A		0.084	0.045					
B					0.039	0.076	0.039	
C					0.022	0.062	0.011	
Means...	0.030	0.074	0.037	0.017	0.030	0.068	0.026	−0.008

Note: Isometric contraction phase averages 0.044 basal and 0.038 after meals. Start of Q to peak of R averages 0.037 basal and 0.042 after meals.

Cardiac output after meals. The basal cardiac indices in this series averaged 2.08 ± 0.19 by acetylene and 2.17 ± 0.26 by calculation (mean deviations). The mean discrepancy between the two estimates on the same occasion was ± 13.1 per cent of the acetylene estimate, if all the data were included. If the cardiac outputs after the meal were similarly compared on a surface area basis, and if, whenever possible, the acetylene value was compared with the mean of the calculated values obtained on either side of it, or vice versa, the mean discrepancy after the meal was ± 11.1 per cent of the acetylene value. A few aberrant acetylene values obviously due to errors have been excluded. The apparent greater error of the basal estimates is due to the inclusion of one value in which both the acetylene and calculated values were aberrant; if this be excluded, the mean discrepancy of the basal series was ± 9 per cent. The mean values for the indices after meals were by acetylene 2.66 ± 0.22 (20 observations)

and by calculation 2.59 ± 0.37 (27 observations); the greater scatter of the calculated values is partly due to the fact that the earliest and latest observations were usually obtained by calculation, so that the times of sampling were more variable. The agreement was, therefore, good. The changes in cardiac output observed, as well as the individual agreement found, may be seen in figures 5 and 6, which represent the data of 9 of the experiments. The heavy line of figure 5 represents experiment 1, in which only calculated values were obtained. The single isolated value not connected with the curves shown at 24 minutes after the *middle* of the meal represents the only occasion on which estimates were attained soon after the meal; in this case observations started 7 minutes after the meal was finished and the high value is probably the result of the movements associated with the eating. The general agreement between this curve and the 5 others on all of the 4 subjects, in which some of the points were determined by acetylene and some by calculation, is striking. The heavy line in figure 6 represents experiment 2, in which all the observations were made by acetylene; it was carried out on the same subject as experiment 1, after a week's interval, at the same time of day and under similar temperature conditions both before and during the experiment. Yet the peak of the change appeared to be higher and earlier than in the other experiment, while the pulse rates were lower, and the curve fits better with the two composite curves obtained on another subject (fig. 6).

Only two experiments are not shown. In one experiment acetylene

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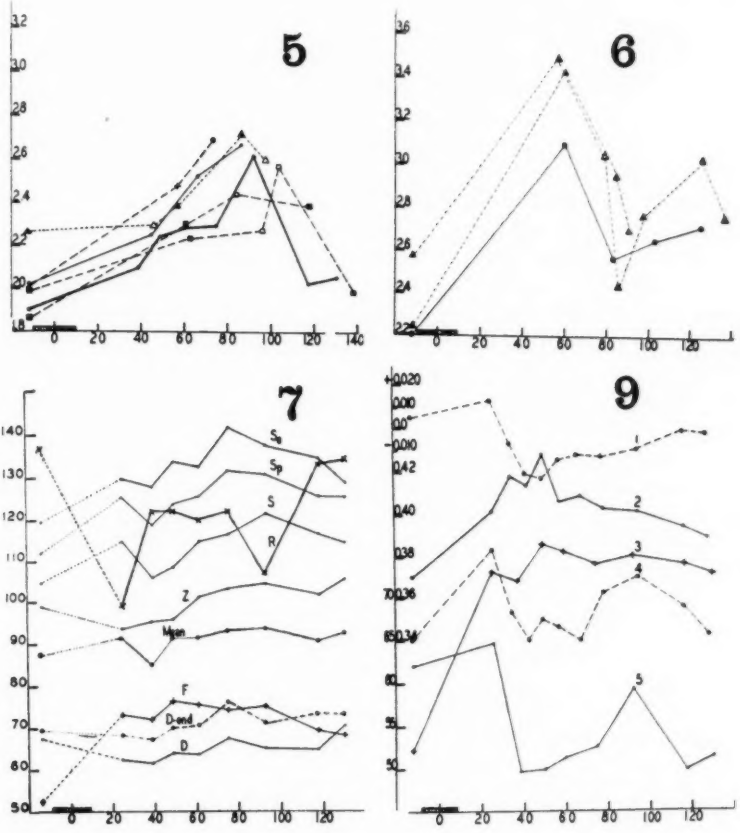
Fig. 5. Cardiac outputs per square meter (ordinates) in relation to time after the middles of the meal in minutes (abscissae). Time of meal indicated by the blocked area. Basal values represent the means of calculated and acetylene values; after the meal calculated values are indicated by solid symbols or a simple cross and, acetylene values by open symbols or a ringed cross. Subject 14 (youngest) + ⊕ subject 4 ▲ △, subject 8 ● ○, and subject 10 ■ □.

Fig. 6. Cardiac output changes of somewhat different type observed on other occasions. Symbols as in figure 5.

Fig. 7. Systolic pressures, *Se*, *Sp*, *S*, the pressure of the dicrotic wave, *Z*, lateral mean pressure, end and lateral diastolic pressure, *De* and *D*, pulse rate *F*, and calculated effective peripheral resistance *R* in experiment 1 as ordinates with time in minutes after the meal as abscissae. All values represent means of 4 or more measurements. (Compare also with table 3 which gives average values.)

Fig. 8. Changes in pulse wave velocity in meters per second and estimated distensibility of the large vessels in experiment 1 as ordinates, and time in minutes as abscissae. Pulse wave velocities *1*, *2*, *3* and *4* (see table 3) are shown in curves of the same numbers. Curve *5* indicates the estimated increase in volume in 0.1 cc. per mm. Hg change in pressure in this subject.

Fig. 9. The time interval end of T wave to 2nd sound in decimals of a second (curve *1*), of the value of the constant K (curve *2*), of the pulse rate (curve *3*) of the constant M^1 (curve *4*) and of the stroke volume (curve *5*) in experiment 1 as ordinates, with time in minutes as abscissae. The scale to the extreme left refers to the pulse rate and stroke volume values.



ERRATUM

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On page 557, figure 5, the single aberrant value referred to in the text has been obliterated by the printer. The value was obtained by calculation and was 2.78 liters per square meter per minute 24 minutes after the middle of the meal.

showed a reduction from a doubtful high index of 2.5 to one of 2.45 and to 2.35 at 55 and at 100 minutes after the meal respectively; calculation values were determined quite independently by another observer and gave indices of 2.15 both before and 75 minutes after the meal. The absence of a rise was indicated by both methods. In the other experiment the basal values were 1.90 and 1.81 by acetylene and calculation; acetylene gave indices of 2.86 and 2.78 at 65 and 118 minutes respectively after the middle of the meal, while calculation gave 3.81 and 3.31 after 96 and 142 minutes. These were the greatest discrepancies ever observed. If the cardiac indices were really as high as those calculated, the acetylene method with samples taken at 14 and 19 seconds would probably have been inadequate to measure it; samples taken at 19 and 23 seconds gave indices 12 to 15 per cent lower, so that the acetylene estimates cannot be considered reliable. The pulse rate increased 32 per cent, and the pulse pressure 42 per cent, changes greater than those usually observed, and the pulse wave velocities increased very little; both factors contributed to the unusually high calculated indices.

Pulse rates, blood pressures, stroke volumes and effective peripheral resistance. The average and extreme values observed for pulse rates and blood pressures are shown in table 3. The *pulse rates* started at the low levels of the basal state (table 4), so that the change in rate after meals was great, and the higher pulse rate was usually maintained at a plateau for 1 to 2 hours. The changes in experiment 1 are shown in figures 7 and 9.

The *blood pressure changes* are also shown in table 3 and in figure 7. Systolic pressure almost invariably rose whatever criterion was used, but if the initial pressures were high, they sometimes fell. Mean pressure varied similarly. The diastolic pressures varied only slightly and in either direction (see fig. 7); in the subjects with the greatest increase in pulse rate diastolic pressure usually rose. Values for the lateral pulse pressures (S-D) are shown separately since the maxima and minima of systolic and diastolic pressures do not refer to the same experiments, and give wrong impressions of the changes in pulse pressure. Pulse pressure was usually increased but might be diminished when the pulse rate change was great.

The differences between end and lateral systolic pressures in individual cases also are not deducible from table 3, but their values are of the same order; they are usually increased. In experiment 1, estimates of differences between end and lateral pressures were extended to include diastolic and mean pressures. The end diastolic pressure (D_e) criterion is not very reliable (Bazett, Laplace and Scott, 1934) but mean end pressures could be deduced from S_e and D_e and from the shape of a pulse curve obtained with an obstruction below the tambour. The end diastolic pressures estimated

are shown in figure 7. The end mean pressures calculated exceeded the lateral by an average of 5.8 mm. (extremes 2.1 and 11.8). No consistent change in the differences between end and lateral mean pressures were found as the result of a meal.

TABLE 3

Average values for all subjects

	F	Se	Sp	S	Z	D	M	S-D	R	PULSE WAVE VELOCITIES			
										(1)	(2)	(3)	(4)
Basal:													
Mean.....	58.7	114.1	109.4	103.9	91.3	65.5	85.5	38.8	119	3.18	5.49	6.63	9.47
Minimum.....	46.2	97.4	93.0	91.5	78.1	55.6	73.9	33.7	88	2.83	4.91	5.37	8.48
Maximum.....	83.1	138.5	128.4	123.2	105.5	83.5	101.7	47.8	164	3.83	7.55	8.79	12.40
After meal:													
Mean.....	78.9	125.6	117.8	109.2	93.3	64.6	87.5	44.7	100	4.04	5.55	7.22	9.91
Minimum.....	56.5	106.2	102.4	95.1	81.5	53.6	79.2	29.5	65	3.03	4.22	6.27	8.25
Maximum.....	109.5	141.4	127.8	121.6	104.3	72.1	96.1	57.7	142	5.60	7.85	9.19	11.69

Se, Sp and S represent end systolic, systolic corresponding to auscultatory, and lateral systolic pressures respectively, Z the dicrotic and D the lateral diastolic pressure. M is mean pressure estimated by curve analysis. Pulse pressure is listed separately (S-D) and R indicates the calculated effective peripheral resistance. Pulse wave velocities are 1, heart to subclavian; 2, subclavian to femoral; 3, subclavian to brachial, and 4, femoral to dorsalis pedis.

TABLE 4

Mean values of stroke volume, etc., by subjects

SUBJECT	CONDITION	PULSE RATE	STROKE VOLUME PER SQUARE METER	M'	K	DISTENSIBILITY PER MM. Hg PER SQUARE METER
						cc.
14	Basal	61	32.2	0.402	0.421	0.68
	After meal	80	38.7	0.400	0.434	0.57
4	Basal	79	29.8	0.422	0.429	0.58
	After meal	102	28.0	0.415	0.445	0.49
8	Basal	49	41.0	0.345	0.379	0.71
	After meal	72	37.1	0.354	0.403	0.54
10	Basal	50	43.1	0.371	0.402	0.94
	After meal	60	39.7	0.353	0.410	0.69

The *stroke volumes* of the different subjects are indicated in table 4. Though a considerable increase in pulse rate was often associated with a

reduction in stroke volume, an increase in stroke volume was indicated by both methods on all subjects in at least one experiment.

The *effective peripheral resistance*, as calculated from the ratio of mean pressure to cardiac index, is shown in table 3 and figure 7. It was reduced after a meal with a single exception.

Pulse wave velocities. The changes observed are shown in table 3 and the values of experiment 1 in figure 8. There was always an increase in pulse wave velocity (1), usually an increase in (3) and (4), while (2) changed little in either direction. Pulse wave velocity (1) was at the extreme upper limit, both for basal and after meal conditions in experiment 1 (fig. 8); the other values did not differ much from average values. The effect of these changes in pulse wave velocity on the distensibility of the whole arterial tree, calculated from the equations previously described (dividing the volumes of the vessels by the squares of the pulse wave velocities, etc.), is also shown in figure 8. The changes in distensibility estimated for the various subjects are shown in table 4. The values indicated in table 4 are the estimated changes in volume in cubic centimeters per millimeter mercury change in pressure, expressed in proportion to the surface area. The apparent high distensibility in the oldest subject is due to the magnitude of the volumes assigned to his large vessels.

Time relations of cardiac cycle. It was found previously that a reduction of stroke volume on standing was usually associated with a reduction in the duration of mechanical systole when expressed in relation to the square root of the cycle. Values of such constants both for the mechanical and electrical changes associated with ventricular systole have been obtained in these experiments; constant M^1 represents the relation of mechanical systole to the cycle (systole $= M^1 \sqrt{\text{cycle}}$) and constant K that of the electrical changes (start of Q to end of T $= K \sqrt{\text{cycle}}$); average values are given in table 4. Changes in M^1 and K in experiment 1 are shown in figure 9, as well as the relation of the 2nd sound to the end of the T wave.

Discussion. Discrepancies observed between estimations of cardiac output by the two methods are explicable as the result of minor technical errors, physiological variations in the subject, or on the incapacity of the acetylene method to evaluate high cardiac outputs. With indices below 3.0 the two methods were in good agreement. The calculation method is valid for these conditions, as far as can be judged by comparison with acetylene. Combined curves obtained by the two methods did not indicate the maintenance of a high plateau of cardiac output as indicated in Grollman's curves (1929). Circulatory changes resulting from the exercise of eating, such as cause the initial alterations in skin temperature reported by Burton and Murlin (1935) were not investigated, except in experiment 1, where there appeared to be an initial high cardiac output followed by a subsidence, with the main rise, like that of surface tempera-

ture, starting some ½ hour to 1 hour and reaching a maximum 1 to 1½ hour after the meal. The character of the curves varied.

The changes in blood pressure, though more completely analysed, resemble those reported by others. The association of a rise in diastolic pressure with a marked increase in pulse rate indicates that the obvious dependence of diastolic pressure on pulse rate may mask the effects of vascular dilatation. Changes in diastolic pressure cannot be taken as necessarily indicating changes in arteriolar tone, which, however, can be approximately estimated by the derived value R. The changes in pulse wave velocity indicate a decrease in distensibility of the large vessels following a meal, a change which is contemporaneous with peripheral dilatation, and may be associated with either a fall or rise of blood pressure. A likely explanation is a constriction of the large vessels, which act as a reservoir and contract when the distensibility of the smaller peripheral vessels is increased by dilatation. Such a reciprocal relationship of the vessels would be in line with their varying reaction to drugs such as histamine (Burn and Dale, 1926). The uniformity of the pulse wave velocity in the abdominal aorta need not imply that this section is not constricted, since the considerable dilatation of the splanchnic area may not be without influence on the wave transmission in the parent vessel.

Certain definite changes in the time relations of cardiac cycle were noted following a meal. In the basal condition the 2nd sound always followed the end of the T wave; after a meal the second sound occurred earlier, and was observed to precede the end of the T wave at some period in all subjects. These changes are indicated in table 2, and figures 4 and 9. The complicated and variable relationship of the 2nd sound to the end of T wave is reviewed by Katz (1928); the reason for a constant change after a meal is not clear. It does not appear to depend simply on the change in pulse rate (fig. 9).

No relationship of stroke volume to the constant K relating the Q-T interval to cycle was observed; this constant was increased, but stroke volume was usually diminished. Variations in the constant M^1, derived similarly from the mechanical changes, also showed considerable disagreement with those in stroke volume if comparison was made before and after a meal; on the other hand, during the period of fast pulse rate, changes in M^1 seemed to parallel those in stroke volume (fig. 9).

CONCLUSIONS

1. Cardiac outputs can be calculated from the changes in blood pressure and pulse wave velocity following a meal with an accuracy of the same order as that obtained under basal conditions.

2. Changes in cardiac output after a meal cannot be represented by a simple plateau curve.

3. The changes in blood pressure and pulse rate are similar to those described by others. The calculated effective peripheral resistance is lowered. Diastolic pressure changes are not a measure of those in effective peripheral resistance. Peripheral dilatation is always associated with a decrease in distensibility of the larger central vessels, as indicated by pulse wave velocities. A constriction of the large vessels is suggested as a possible cause, and presumably these vessels act as an adjustable reservoir.

4. A method of recording sternal movements is described, and is utilised for the timing of the start of cardiac ejection. The relationship of such curves to electrocardiograms is discussed.

5. The time relations of the electrical and mechanical changes so measured before and after meals are described.

6. A source of error in the preservation of acetylene samples over mercury is mentioned.

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ON THE ADAPTIVE SECRETION OF THE GLANDS OF THE JEJUNUM[1]

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Most of the glands of the digestive tract, including those of the small intestine, have been studied to determine whether their enzyme production is adapted to changes in diet. The work of Simon (1907), Neilson and Lewis (1908) and Evans (1913) with human subjects showed that high carbohydrate meals evoked the secretion of saliva containing much amylase. In his review of the work of the Pavlov school Babkin (1928) states that the production of pepsin may be quite variable even after meals of protein so closely related as horse meat and veal. Furthermore starch was found to evoke gastric juice rich in pepsin. Babkin (1928, p. 488) and Baxter (1935) have shown that pancreatic amylase, trypsin and steapsin are secreted in the same relative amounts regardless of the stimulus used to excite the pancreas.

London and Krym (1910) and London and Dobrowolskaja (1910) introduced chyme and different foodstuffs directly into fistulae of the intestine. The total amounts of protease, amylase, and lipase secreted in response to such local stimuli varied considerably whereas the relative amounts remained nearly constant. TeGroen (1914) introduced starch paste into Thiry-Vella loops and found that the amylase content of the resulting secretion was twice as great as the control. London (1914) pointed out that this type of experiment proved only that the intestine responds to local stimulation and not that amylase is preferentially secreted in excess of the other enzymes. The same criticism holds for the work of Jansen (1910) who determined only lipase activity of succus entericus obtained after local stimulation by fat and bile. Plimmer (1906) attempted, without success, to increase the lactase content of intestinal mucosa by feeding milk and lactose. Andrejew and Georgiewsky (1932), (1934) and Georgiewsky and Andrejew (1935) concluded that a high carbohydrate diet brought about the secretion of intestinal juice especially

[1] The data in this paper are taken from a thesis presented by T. L. Bourns to the Graduate School, University of Rochester, in partial fulfillment of the requirements for the degree Master of Science.

rich in amylase. Since no other enzyme was determined it is impossible to tell from their results whether amylase was the only enzyme which was increased. Furthermore, the collection period of 1½ hour was too short to include the maximum response to a meal, which usually is attained about 3 or 4 hours after feeding (Nasset, Pierce and Murlin, 1935).

The foregoing brief review indicates some differences of opinion on the subject of adaptation to diet by the glands of the digestive tract. The work on the intestine has been especially confusing because of inadequate methods and control experiments. In establishing proof for a humoral mechanism in intestinal secretion, a technic was developed for studies of transplanted segments with and without the mesenteric nerves intact (Nasset, Pierce and Murlin, 1935). It was shown that during digestion the extrinsic nerves of such transplants often exerted an inhibitory influence on the secretion of enzymes. This fact accounts for the variable responses to feeding so characteristic of the Thiry-Vella type of fistula. Apparently previous experiments on the adaptation of intestinal glands to diet were made with this type of fistula. It seemed desirable, therefore, that the subject should be reinvestigated, using intestinal segments both before and after division of the extrinsic nerves.

METHODS. *Experimental animals.* Bitches with functionally hypertrophied mammary glands were selected so that the jejunal segments transplanted to this vascular region might readily develop a collateral circulation. The transplants, closed at one end, opened on the skin of the abdomen. The *first stage* operation involved only the embedding of a piece of intestine, with mesentery intact, on either side of the midline. About six months later a *second stage* operation, in which the mesenteric pedicle was cut, demonstrated that the collateral circulation was adequate to maintain the transplants. In none of the 7 *second stage* dogs that have come to autopsy since 1932 has there been any direct vascular connection between transplants and mesentery.

Diet. The basal ration in most experiments was a commercial dog biscuit, referred to as "chow" in the tables. By some preliminary trials it was determined about how much the dogs would eat and then this amount was fed throughout the experimental period. At times the food was not all consumed but the body weights of the animals were maintained, with two exceptions which will appear later.

Two *second stage* dogs were kept for 25 weeks on a diet of "chow." From time to time as indicated in table 1, fresh baker's yeast[2] (26 gm.) was added to the diet to determine whether the vitamin B complex had any effect on intestinal secretion. It became evident after the first yeast period that the secretion was increased. Vitamins B and G were destroyed

[2] Fleischmann's baker's yeast kindly supplied by Standard Brands, Incorporated.

in the next yeast supplement by autoclaving for 6 hours at 120 to 124°C. and pH 9 to 10 (Chick and Roscoe, 1930) (Williams, Waterman and Gurin, 1929). There could be no doubt about the destruction of vitamin B; the destruction of vitamin G was demonstrated according to the method of Bourquin and Sherman (1931). The total N fed in the fresh and autoclaved yeast supplements remained the same in all periods with the exception of a two-week period in which the amount of autoclaved yeast was doubled. Throughout the 25 week period, the total secretion of 3 typical enzymes (sucrase, amylase and peptidase) of intestinal juice was determined quantitatively for each collection.

After the "chow"-yeast regime the dogs were kept for 3 successive periods of 3 weeks each on diets of potato (cooked, peeled and mashed), beef heart freed of visible fat, and 40 per cent cream respectively. These diets were fed in isocaloric quantities.

In order to mitigate the severe conditions imposed by a regimen such as described in the preceding paragraph, a third set of experiments was carried out in which "chow" furnished three-fourths of the calories and corn starch, beef heart or 40 per cent cream supplied the remaining one-fourth. These diet periods ran 2 weeks. In the second week of each period the usual supplement of baker's yeast was added.

Chemical. The 7 hour collection of juice was begun about an hour after feeding (8:00 a.m.) the volume recorded hourly and the juice placed in the cold room until needed. Enzyme determinations were made according to procedures described in detail elsewhere (Pierce, Nasset and Murlin, 1935). Direct quantitative determinations were made with 1.0 cc. of juice incubated for 48 hours at 38°C. This value was multiplied by the average hourly volume of juice to give total enzyme production per hour.

Total solids were obtained by drying 1.0 cc. of juice to constant weight at 105°C. The ash was determined by heating this dried material in a muffle furnace for 20 minutes at 600°C.

RESULTS. Table 1 summarizes the work of 25 weeks with 2 *second stage* dogs on the basal ration of dog biscuit with yeast supplements. The control periods were alternated with the yeast periods as indicated by the dates but in the table they are grouped separately to facilitate comparison of averages. The difference between the rates of secretion on fed and on fasting days is the "food effect" and is expressed as a percentage of the fasting value. A vertical comparison gives the "yeast effect." It is obvious that the addition of yeast, whether fresh or autoclaved, resulted in an increased secretion of intestinal juice.

It not only produced a relatively better response to feeding but also increased the absolute amount of secretion on both fasting and fed days. The fact that the effect was carried over to fasting days indicates perhaps that some conditioning process occurred which persisted for a time in

the absence of an immediate supply of yeast. It should be noted that with one exception (dog 6) responses on fed days in the yeast periods were always better than either adjacent control. Boldyreff (1931) increased

TABLE 1

The rate of secretion from denervated jejunal transplants as influenced by yeast supplements to the diet

DIET	VOLUME OF JUICE PER HOUR		NUMBER OF EXPERIMENTS AVERAGED		DOG NUMBER
	Fasting	Fed	Fasting	Fed	
	cc.	cc.			
Chow 12–11/1–9	3.2	7.2	6	6	6
Chow 2–7/3–6	3.8	9.2	6	6	
Chow 4–18/5–15	3.7	10.1	6	6	
Average	3.6	8.8			
Food effect, per cent		+144			
Chow + 2 cakes yeast/day 1–10/2–6	3.8	10.3	6	6	6
Chow + autoclaved yeast 7 gm. 3–7/4–3	3.5	11.6	6	6	
Chow + autoclaved yeast 14 gm. 4–4/4–17	5.5	12.1	3	3	
Chow + 2 cakes yeast/day 5–16/6–5	3.9	10.0	5	4	
Average	4.2	11.0			
Food effect, per cent		+162			
Yeast effect, per cent	+17	+25			
Chow 12–11/1–9	2.6	4.0	6	6	8
Chow 2–7/3–6	3.0	5.1	6	6	
Chow 4–18/5–15	4.8	6.3	6	6	
Average	3.5	5.1			
Food effect, per cent		+46			
Chow + 2 cakes yeast/day 1–10/2–6	3.6	5.3	6	6	8
Chow + autoclaved yeast 7 gm. 3–7/4–3	3.9	7.5	6	6	
Chow + autoclaved yeast 14 gm. 4–4/4–17	3.6	8.2	3	3	
Chow + 2 cakes yeast 1 day 5–16/6–5	4.6	9.0	5	4	
Average	3.9	7.3			
Food effect, per cent		+87			
Yeast effect, per cent	+11	+43			

the secretion of Thiry-Vella loops by irrigating them with yeast extracts. As mentioned above, enzyme determinations were made on each day's juice but there were no consistent changes in enzyme production as a result of yeast feeding. In order to save space these results were omitted.

If adaptation of enzyme production to diet occurs in this type of experiment, it should be observed on diets rich in protein, carbohydrate or fat. Therefore, the *second stage* dogs (6 and 8) and a pair of *first stage* dogs (9 and 10) were placed on the second dietary regime described under methods. Dogs 6 and 8 lost weight on the potato diet so that it was necessary to give them a two weeks' rest on hospital scraps before feeding them beef heart. They were not put on the cream diet. A summary of these experiments is presented in table 2. Each figure in this table is the mean of 3 determinations made on different days.

TABLE 2

Effect of various dietary regimes on the quantity and quality of intestinal juice

DIET AND DOG NUMBER	VOLUME OF JUICE PER HOUR		SUCRASE* (INVERT SUGAR)		AMYLASE (GLUCOSE)		PEPTIDASE (AMINO N)		LIPASE (FATTY ACIDS)	
	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting	Fed
	cc.	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Dog 6 (2nd stage)										
Potato 6-13/7-3.....	4.2	4.8	834	1006	847	773	88	96	13	11
Food effect, per cent....		+14		+21		−9		+9		−15
Beef heart 7-25/8-14....	4.0	6.4	1482	1975	1386	1749	128	158	29	35
Food effect, per cent....		+60		+33		+26		+23		+21
Dog 8 (2nd stage)										
Potato 6-13/7-3.....	3.0	3.3	182	183	334	426	78	60	7	9
Food effect, per cent....		+10		0		+28		−23		+29
Beef heart 7-25/8-14....	4.2	5.0	265	176	551	576	98	113	16	19
Food effect, per cent....		+19		−34		+5		+15		+19

* The enzyme value is the 48 hour activity of 1 cc. of juice multiplied by the average hourly volume of juice secreted over a period of 7 hours.

The data of table 2 give no evidence of adaptation to starch feeding. Although the amylase showed a good increase in dog 8, on fed days, the production of this enzyme was actually decreased by feeding in dog 6. Furthermore, the greatest relative increases on fed days occurs with sucrase and lipase in dogs 6 and 8 respectively. The same general relations are true for the high protein diet. The outstanding difference between the potato and the beef heart diet is that the absolute amount of enzyme produced is much greater, with one exception, on the latter diet.

Table 3 shows the results obtained with two *first stage* dogs (9 and 10). One outstanding fact is the inhibitory effect of feeding on enzyme production in two-thirds of the experiments. There is only one instance of de-

creased volume of juice after feeding. These results confirm the earlier ones and support the hypothesis expressed before (Nasset, Pierce and Murlin, 1935) that the extrinsic nerves of the intestine inhibit the production of succus entericus in portions of the intestine not in contact with chyme. If adaptations to diet were mediated by way of reflexes, one might expect to demonstrate the process in first stage dogs where the mesenteric nerves were not disturbed. For this purpose one cannot compare fasting with fed days because of the inhibition noted above. In

TABLE 3

Effect of various dietary regimes on the quantity and quality of intestinal juice

DIET AND DOG NUMBER	VOLUME OF JUICE PER HOUR		SUCRASE (INVERT SUGAR)		AMYLASE (GLUCOSE)		PEPTIDASE (AMINO N)		LIPASE (FATTY ACIDS)	
	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting	Fed
	cc.	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Dog 9 (1st stage)										
Potato 6-13/7-3.....	2.3	3.0	747	924	770	911	60	60	7	8
Food effect, per cent....		+30		+24		+18		0		+14
Beef heart 7-4/7-24.....	2.9	4.5	807	508	1096	903	80	83	4	3
Food effect, per cent....		+55		−37		−18		+4		−25
Cream 7-25/8-14.....	3.7	4.6	458	95	806	596	86	47	3	1
Food effect, per cent....		+24		−79		−26		−45		−67
Dog 10 (1st stage)										
Potato 6-13/7-3.....	2.5	3.5	895	928	825	923	66	55	8	6
Food effect, per cent....		+40		+4		+12		−17		−25
Beef heart 7-4/7-24.....	5.9	7.2	1893	1843	1872	1893	117	181	19	16
Food effect, per cent....		+22		−3		+1		+55		−16
Cream 7-25/8-14.....	7.0	6.8	2221	1602	1875	1213	161	132	34	19
Food effect, per cent....		−3		−28		−35		−18		−44

comparing the results on one diet with those of the others, it is seen that there is very little evidence of adaptation. In both dogs the fasting secretion of juice is greatest on cream. After feeding the volume is greatest for cream and beef heart in dogs 9 and 10 respectively. The enzyme output is typically variable and gives no evidence that the secretion of any single enzyme is favorably or adversely affected by any particular type of diet. The only result which lends support to the adaptation idea is the peptidase production on beef heart. In both dogs the response to a meal of meat is positive and also greater than to a meal of either potato or

TABLE 4

Effect of various dietary regimes on the quantity and quality of intestinal juice

DIET AND DOG NUMBER	VOLUME OF JUICE PER HOUR		SUCRASE (INVERT SUGAR)		AMYLASE (GLUCOSE)		PEPTIDASE (AMINO N)		LIPASE (FATTY ACIDS)	
	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting	Fed
	cc.	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Dog 9 (2nd stage)										
Chow 9–19/10–2	3.1	3.4	509	648	773	961	63	74	9	8
Food effect, per cent.		+10		+27		+24		+17		−11
Chow + beef heart 10–3/10–16	3.3	5.1	798	1020	1189	1508	73	97	12	14
Food effect, per cent.		+55		+28		+27		+33		+17
Chow + starch 10–17/10–30	3.1	3.6	1374	1112	1159	1253	95	78	23	22
Food effect, per cent.		+16		−19		+8		−18		−4
Chow + cream 10–31/11–13	2.5	3.2	906	1294	983	1252	53	109	22	30
Food effect, per cent.		+28		+43		+27		+101		+36
Chow 11–14/11–27	1.8	2.5	835	945	781	986	80	84	21	23
Food effect, per cent.		+39		+13		+13		+5		+10
Dog 10 (2nd stage)										
Chow 9–19/10–2	2.3	5.5	991	1649	814	1337	63	103	15	15
Food effect, per cent.		+139		+66		+64		+64		0
Chow + beef heart 10–3/10–16	3.0	4.5	1337	1743	1065	1589	95	109	17	21
Food effect, per cent.		+50		+30		+49		+15		+24
Chow + starch 10–17/10–30	2.7	6.3	1062	2424	1055	2060	77	152	21	38
Food effect, per cent.		+133		+128		+95		+98		+81
Chow + cream 10–31/11–13	2.4	5.2	1009	1801	960	1535	71	122	22	34
Food effect, per cent.		+117		+79		+60		+72		+55
Chow 11–14/11–27	2.5	3.7	1116	1507	973	1466	88	99	23	29
Food effect, per cent.		+48		+35		+51		+13		+26

cream. On the other hand, a favorable effect may be noted also on the secretion of the other enzymes.

The *second stage* operation was performed on dogs 9 and 10 September

11, 1935. Eight days later they were placed on diet as indicated in table 4. The carbohydrate, fat and protein were given as indicated under methods. The greatest volume of juice was secreted on beef heart and starch supplements in dogs 9 and 10 respectively. Again it is evident that the type of diet does not affect the relative proportions of the 4 enzymes which were determined. The fallacy of drawing conclusions from the determination of a single enzyme is obvious. Sucrase and amylase in dog 10 are higher on the starch rich diet than on any other, but the same is true for peptidase and lipase. The outstanding feature of these data is the preponderance (90 per cent) of positive responses to feeding. This is quite different from the results for the same dogs *first stage* (table 3).

TABLE 5

The effect of adding a cream supplement on the solids and ash of intestinal juice

DIET* AND DOG NUMBER	VOLUME OF JUICE PER HOUR		TOTAL SOLIDS		ASH		ALKALINITY OF THE ASH			
	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting		Fed	
	cc.	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*m.eq./ cc.*	*m.eq./ hr.*	*m.eq./ cc.*	*m.eq./ hr.*
Dog 9 (2nd stage)										
Chow + cream.....	2.5	3.2	3.80	4.62	0.85	0.90	0.035	0.088	0.037	0.118
Chow.....	1.8	2.5	5.07	4.56	0.76	0.89	0.021	0.038	0.025	0.063
Dog 10 (2nd stage)										
Chow + cream.....	2.4	5.2	3.94	3.23	0.86	0.93	0.020	0.048	0.024	0.125
Chow.....	2.5	3.7	4.02	3.72	0.82	0.86	0.025	0.063	0.024	0.089

* Each dietary regime was continued 2 weeks. Thus each figure in the table is the average of 3 determinations.

It was mentioned above that fresh baker's yeast was given as a supplement in the last week of each diet period, represented in tables 2, 3 and 4. It was found that a week's yeast feeding was insufficient to produce any consistent change in the volume of juice secreted and, therefore, the results for any diet appear as an average for the entire period.

The organic matter of the juice was not expected to show any consistent change on different diets because the enzyme production failed to do so. However, there remained the possibility that the inorganic components might show some variation with diet. The data on this point are not so extensive as for enzyme production but probably sufficient to show that the concentration and alkalinity of the ash does not vary greatly with diet (table 5).

There is a periodicity in the secretion of isolated intestinal segments

(Boldyreff, 1928) (Bickel and Wagner, 1934) (Nasset, Pierce and Murlin, 1935). The mean hourly response of dog 10 on various diets is depicted in figure 1. These curves were obtained by properly grouping the individual experiments presented in summary in table 4. The curves for dog 9 are quite similar with two exceptions, which are: *a*, the magnitude of the response to food is usually less, and *b*, the curve for the cream diet is almost a mirror image of the one shown for dog 10.

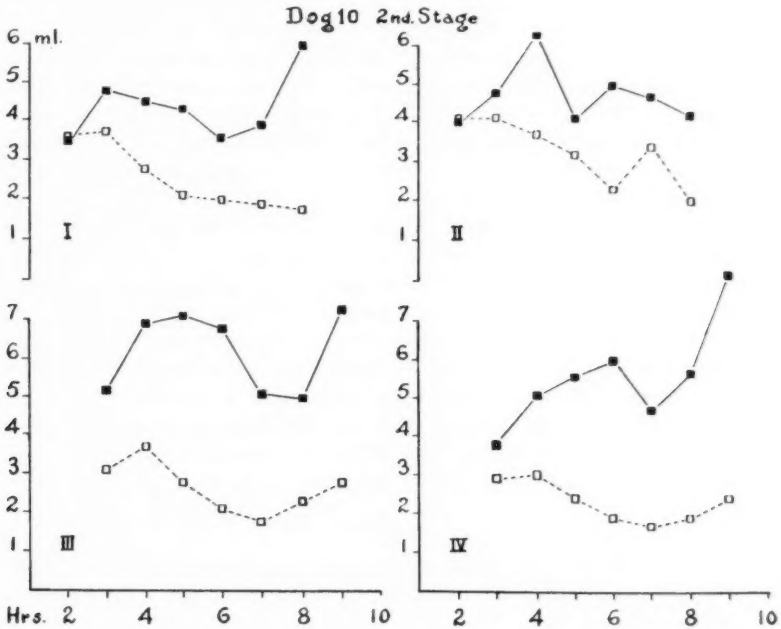

Fig. 1. Secretion of intestinal juice. Solid squares—fed; open squares—fasting (24 hours). Abscissa shows time after feeding. I, "chow" 6 (experiments). II, "chow" + beef heart (3 experiments). III, "chow" + starch (3 experiments). IV, "chow" + cream (3 experiments).

COMMENT. The criterion of adaptation which we have chosen is that the enzyme most needed in the digestion of a certain type of diet shall be secreted in significantly greater or less proportion than the other enzymes. These two conditions are included because conceivably there may be either a direct or a reciprocal relation between the normally functioning gut and an isolated segment. On the one hand a stimulus to secrete a certain enzyme in excess might excite the glands regardless of position, but on

the other hand hypersecretion of any enzyme into the intestine in response to local stimuli might lead to a corresponding impoverishment of the juice secreted by an isolated segment. In order to determine whether either of these conditions was satisfied, it was necessary to know the behavior of intestinal transplants when the animals were on a constant diet. This information was supplied by dogs 6 and 8 on the "chow"-yeast regimen. In the 3 four-week control periods these dogs were fed exclusively on dog biscuit so that the enzyme production as observed may be taken as typical of the response to a constant diet. The enzyme production of these dogs on potato or beef heart (table 2) was not qualitatively different from that found on the "chow" diet. That is, of 3 enzymes (sucrase, amylase and peptidase) no single one was secreted in significantly greater or lesser amount than the other two. Since these results were obtained on *second stage* dogs, it appears that there is no adaptation traceable to humoral factors. This conclusion is supported also by the data, for dogs 9 and 10 *second stage*, shown in table 4.

The possibility of adaptation effected by reflexes including the extrinsic nerves is ruled out by the results presented in table 3. There are exceptions but these are to be expected in light of the erratic responses noted even on a constant and complete control diet. The possibility of adaptive secretion occurring locally due to the peculiar chemical make-up of chyme has not been investigated, the chief difficulty being the simultaneous quantitative determination of several enzymes in such a mixture. However, the work of London and Lukin (1910) on this phase of the problem indicates that at least single local applications of various food mixtures are incapable of changing the quality of the intestinal juice.

SUMMARY

1. The secretion from isolated intestinal segments, before and after denervation, was examined for enzyme activity and volume under a variety of dietary regimes.

2. The addition of small supplements of baker's yeast increased the quantity of juice secreted. This effect of yeast persisted after the destruction of vitamins B and G.

3. Diets rich in carbohydrate, protein or fat failed to alter significantly the relative concentrations of several enzymes of the intestinal juice.

4. It is concluded that there are no humoral or extrinsic nervous factors which operate to change the relative concentrations of the enzymes studied during a three-week feeding period.

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THE EFFECT OF PIPERIDINOMETHYLBENZODIOXANE (933F) AND YOHIMBINE UPON THE ACTION OF CERTAIN DRUGS AND IONS ON THE NICTITATING MEMBRANE

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Piperidinomethylbenzodioxane (933F) decreases only slightly the contractile responses of the nictitating membrane (n.m.) of the cat to nervous excitation, while the responses to adrenalin and sympathin are markedly diminished (Bacq and Fredericq, 1935a, b). Yohimbine has been observed to exert a similar differential effect upon the responses of smooth muscle to adrenalin, sympathin and nervous excitation (Bacq, 1936; Rosenblueth and Cannon, 1936).

The present work was undertaken to determine whether 933F and yohimbine depress specifically the responses of the n.m. to adrenalin and sympathin, or whether the depression is extended to other substances ordinarily stimulating contraction.

METHOD. Cats were used, under dial anesthesia (0.8 cc. per kgm.). The right n.m. was sensitized by removal of the right superior cervical ganglion at least six days previous to the experiments, and by the intravenous injection of cocaine (8 mgm. per kgm.). Isotonic contractions of this membrane were recorded. Both adrenal glands were ligated. Artificial respiration was employed to eliminate the effects of asphyxia. Injections of varying dosages of adrenalin (1 to 10 γ), acetylcholine (10 to 100 γ), $CaCl_2$ (25 to 75 mgm.) and KCl (25 to 75 mgm.) were made into the femoral vein, before and after intravenous administration of 933F (3 to 4 mgm. per kgm.), or of yohimbine (3 to 4 mgm. per kgm.).

RESULTS. 1. *933F.* The contractile responses of the n.m. to adrenalin, acetylcholine, $CaCl_2$ and KCl were markedly decreased in magnitude after administration of 933F (fig. 1). Quantitatively this decrease was not as great in the case of acetylcholine as with the other substances studied. The decrease of the responses to K and Ca was approximately as great as that of the responses to adrenalin.

2. *Yohimbine.* Similar results were obtained following the injection of yohimbine—depression of responses to all the substances being observed. The depression of the action of acetylcholine was again less than that of the other substances.

DISCUSSION. Monnier and Bacq (1935) have cited the differential effect of 933F on the responses of the n.m. to adrenalin and to nerve stimulation as evidence of the existence of both electrical and chemical mediation of the nerve impulses in smooth muscle. They believe that 933F blocks the response to the adrenine-like chemical mediator, without affecting the electrical transmission.

Rosenblueth and Cannon (1936) have offered evidence opposed to the existence of two mechanisms of transmission, and have concluded that

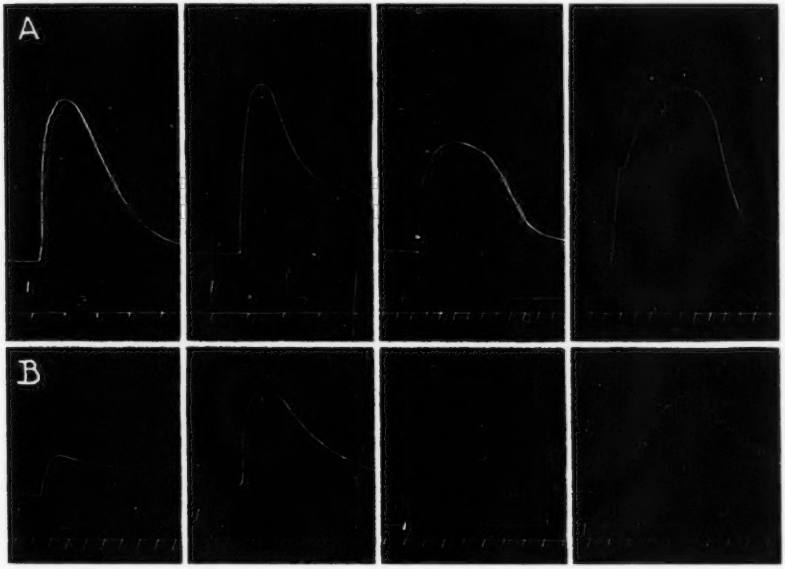

Fig. 1. Dial. Isotonic contractions of the nictitating membrane, sensitized by denervation 6 days previously and by intravenous injection of cocaine (8 mgm. per kgm.). Adrenals ligated. Time recorded in 30 second intervals. Effects of injecting: adrenalin (1 γ), acetylcholine (10 γ), $CaCl_2$ (25 mgm.) and KCl (25 mgm.). A before, and B after 933F (3.5 mgm. per kgm.).

the observed phenomena can be explained on a basis of purely chemical mediation. They cite facts indicating that 933F and yohimbine increase the polarization of the cell membrane, thereby making it less permeable to adrenine and decreasing the cell responses to this agent. The action of sympathin liberated intracellularly as a result of nerve stimulation would not be affected by this variation in membrane permeability, although there would be a decrease in the diffusion of the hormone to other cells and a resultant decrease in the total response of the membrane to nerve stimulation, since not all the smooth muscle cells receive a nerve supply.

If the suggestion made by Rosenblueth and Cannon is valid, the decreased permeability of the cells should probably not be specific for adrenine or sympathin, but should extend to other substances as well. The present results support their suggestion, since the responses to all the substances tested were depressed (fig. 1). A ready explanation for the quantitative differences encountered is not available.

SUMMARY

933F and yohimbine decrease the contractile responses of the nictitating membrane to acetylcholine, $CaCl_2$ and KCl, as well as to adrenalin and sympathin.

The depression of the response to acetylcholine is quantitatively less than that observed with the other substances studied.

The significance of these results in relation to the mode of transmission of the nerve impulse in smooth muscle is discussed.

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THE EFFECTS OF ANESTHETICS ON ACTION POTENTIALS IN THE CEREBRAL CORTEX OF THE CAT

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The action potential is the most direct index of activity in the nervous system. This familiar fact has been applied to the study of the cerebral cortex in numerous recent researches, with the use of modern methods of amplified electrical recording. A number of workers have given interesting accounts of the cortical potentials occurring under both local and general anesthesia (for bibliography see Berger, 1929; Fischer, 1932; Kornmüller, 1935; Jasper and Andrews, 1936; Gibbs, Davis and Lennox, 1935); but a systematic study of the effects of anesthetics on cortical potentials, particularly in relation to the normal type from the unanesthetized animal, has not yet been reported. The present research was designed to obtain information on the mode of action of various anesthetics on the central nervous system, as indicated by their effects on cerebral action potentials and on reflex activity, and, if possible, through this means to throw light on the nature of the nervous mechanism itself. For comparison, a few studies were made on unanesthetized preparations.

Electrical potentials were recorded from different parts of the cerebral cortex of the cat during spontaneous activity and under sensory stimulation, under varying depths of anesthesia induced by ether, avertin or pentobarbital sodium. These anesthetics were chosen because they represent a volatile, an alcoholic and a barbituric type, and because all three are widely used in clinical surgery.[1]

METHOD. With the animal under surgical anesthesia, recording electrodes were placed on the cortex. These electrodes were L-shaped silver plates, insulated with ambroid except for one surface, 4 mm. square, which was chlorided. The electrodes were slid under the bone, with the chlorided surface in contact with the dura, and were then cemented in place with bone wax, thus leaving no part of the cortex exposed. The body temperature of the animal was kept nearly constant by artificial heating. The

[1] A preliminary report of our earlier experiments has appeared (Forbes, Derbyshire, Rempel and Lambert, 1935). Since that report was made we have extended the observations with many more experiments, which enable us to make more definite generalizations.

uniformity of the results tends to show that the conditions of moisture and temperature in the cortex remained fairly constant throughout the experiment.

Our standard procedure was to place the differentiated electrodes on selected areas of the right cortex and one grounded electrode on the left motor cortex, which had been extensively cauterized. The purpose of cauterizing the cortex on the opposite hemisphere to that whose activity was being studied was to simplify the conditions of recording, as far as possible. If the two electrodes are placed on two active groups of cells, the electrical record tends to be a confusing composite of the activities of the two groups. We hoped that by rendering inactive all of the cortical tissue in the vicinity of the "indifferent" electrode, we could minimize this source of confusion. Dusser de Barenne and McCulloch (1935, 1936) have shown that as successive layers of the cortex are destroyed by heat the waves described by the above-mentioned investigators progressively disappear. This seems to show that they are due to activity in the cortical gray matter. We have controlled the effect of cautery in some experiments by leading off from the burnt cortex to the muscles of the neck. When the cortex was deeply burned, the record showed only very small excursions, much smaller than those which characterized the response of the intact cortex. This control indicated that the cautery had served its purpose. A selector switch in the grid circuit permitted the use of each lead on the active cortex separately. Since we were dealing with the effects of sensory stimulation, especial attention was given to the sensorimotor area of the hind leg. The motor area was determined by electrical stimulation in each experiment.

The potential changes between the ground lead and one of the active leads were amplified by a resistance-capacity coupled amplifier (Garceau and Forbes, 1934), and recorded with a Hindle string galvanometer. With the application of a constant potential to the amplifier, the excursion of the string falls to one-half its initial value in 12 seconds. The system will consequently deal efficiently with frequencies as low as 1 cycle in 12 seconds. The upper frequency limit of efficient recording is determined by the characteristics of the string. In our experiments, three strings were used, with resistances varying from 3000 to 9000 ohms. They were used at slack tensions, such that the application of a constant current gave an excursion which was complete in about 20 milliseconds (msec.); consequently the distortion in recording frequencies up to 70 per second was slight.

In a few experiments records were made with the amplifier described by Garceau and Davis (1934) feeding into the undulator, the cathode ray oscillograph, or both. Examples taken by the three recording devices on the same animal under similar conditions are shown in figure 1. A com-

parison of these records will show that all three record with approximate fidelity all of the major waves. Some of the more rapid waves do not appear in the undulator records, but are brought out in the records with the cathode ray and with the string galvanometer. A few records were made with the unaided string galvanometer, high sensitivity being attained by the use of a slack string.

To obtain sensory stimulation, the central end of the entire left sciatic nerve was placed in tube electrodes and connected with a stimulating circuit consisting of a key, a 1.5-volt dry cell, a string-galvanometer signal

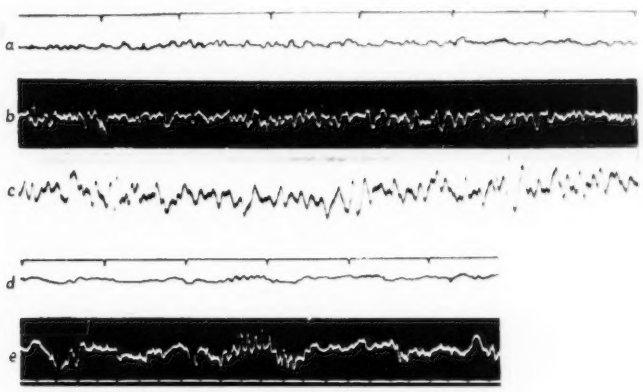

Fig. 1. Records of spontaneous action potentials from unanesthetized motor cortex in two preparations, to show a comparison of three recording instruments. All reproduced on approximately the same time scale.

a, b and c are from one animal on the same day under similar conditions. d and e, from another animal, were taken simultaneously with the two recording devices connected with the same amplifier. Individual waves can be identified and compared. a and d, ink-writing undulator, overdamped (time above in seconds); b and e, cathode ray oscillograph (time below in $\frac{1}{4}$ sec.); c, string galvanometer (time below in $\frac{1}{25}$ sec.).

device (Forbes and Cattell, 1924), a balanced Bishop coil (Bishop, 1927) and a rotary interrupter (Forbes, 1921). The frequency of stimulation (counting both make and break shocks) was usually 140 per second. The homolateral hamstring nerves were kept intact in order that the flexion reflex could be recorded on a smoked drum as an indication of the depth of anesthesia. Unless otherwise stated, a strength of stimulation which evoked a maximal flexion reflex was used.

For the study of the cortical potentials of unanesthetized cats, small rectangular silver plates soldered to slender bolts were used as electrodes. With the animal under deep anesthesia and with aseptic precautions, these

plates were inserted into rectangular openings in the skull and were rotated 90°. The remainder of the rectangular hole was filled with bone wax. A Bakelite washer was placed over the bolt next to the surface of the skull and held firmly in place with a nut. Two such electrodes were applied—the grounded lead over a large cauterized area of the parietal region, and the differentiated electrode over a selected region of the opposite motor cortex. The position of this electrode is fixed by the pressure between the silver plate on the inside of the skull and the washer and nut on the outside. The surfaces of the bolts and the silver plates in contact with the bone had been previously insulated with a baking varnish. After application of these electrodes, the surfaces of the bolts, nuts and washers which were in contact with the skin and subcutaneous tissue were painted with a coat of collodion solution. The scalp was then closed with blind stitches, allowing the bolts to stick out through the skin. The entire wound was covered with a sterile gauze bandage, held in place with Duo Liquid Adhesive, allowing the bolts to project through the bandage.

The cat recovered usually without any signs of irritation from the electrodes. Only one out of three made any attempt to scratch at them. The wound usually remained without evidence of infection for about five days after the operation. Leads from the amplifier were secured to a harness on the shoulders of the cat and from there made contact with the bolts by means of flexible wires. This arrangement allowed free motion of the head without disturbance of the contacts.

Ether was administered by a tracheal cannula with a by-pass tube for dilution with air. Avertin[2] and pentobarbital sodium were both injected intraperitoneally. For surgical anesthesia we used a dosage of 100 mgm. of avertin per pound of body weight. In the case of pentobarbital sodium we used 15 to 20 mgm. per pound. For extra deep anesthesia we added from 40 to 100 per cent of the initial dose.

Results. In spite of the complex behavior of the cortex, the basal patterns of activity and the responses to sciatic stimulation show certain characteristics which may be found in almost all cats. Such variability as is found may be in part due to the wide range in depth of anesthesia and in part to individual differences. Topographical differences in the spontaneous pattern and in responses to stimulation of the sciatic nerve are apparent in the comparison of different regions of the cortex, but we did not make a complete study of their distribution.

Since the majority of our records were made with the "active" lead on the motor cortex, we shall first describe the results thus obtained. We shall then deal with such evidence as we found relating to the differences between different parts of the cortex.

[2] The avertin (tribromethanol in amylene hydrate) was generously furnished by the Winthrop Chemical Company.

I. Unanesthetized Cats. In the unanesthetized cats studied thus far, the differentiated lead was placed posterior to the cruciate sulcus and anterior to the sulcus ansatus. When the animal is lying quietly with

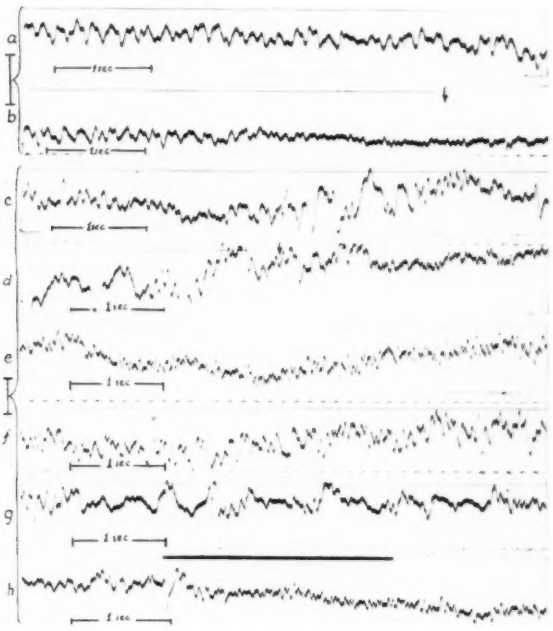

Fig. 2. Records from two animals aseptically prepared for study of unanesthetized motor cortex. In this and all subsequent figures, the records were made with the string galvanometer; upward excursion signifies negative potential of "active" (grid) lead.

a, 5 days after operation. Unanesthetized animal at rest. b, same animal during spontaneous stirring. Arrow shows time when observer noted turning of head. c-h, another animal. c, 3 days after operation, animal asleep. d, same day, animal aroused from sleep by calling; approximate time marked by arrow. e, next day, 4 minutes after injection of avertin, animal active. f, 2 minutes after e, anesthesia becoming effective. g, 15 minutes after injection, anesthesia fairly deep. h, 3 hours later, after additional dose of avertin and application of stimulating electrodes to sciatic nerve. Duration of stimulation shown by heavy signal line above record.

Sensitivity shown by vertical lines at the side representing 100 microvolts. Each line applies to group of records shown in brackets.

eyes partially closed, the cortical potentials may show a dominant pattern of about 6 waves per second with an amplitude of about 60 microvolts. These waves do not have smooth contours (fig. 2a), but have superimposed

upon them smaller excursions at about 14 per second. These large waves are not always as regular as they appear in this record. Correlated with gross spontaneous muscular movements or responses to sensory stimulation or even with the appearance of attentiveness, there was usually a break in the slower rhythm and a change to much smaller waves of higher frequency. This effect was produced by tactile stimuli (pinching the foot) and by various acoustic stimuli.

An example of the activity recorded during a spontaneous movement (the sudden turning of the cat's head) is shown in figure 2b. The approximate time was recorded by the manually operated signal just after the motion was observed. During the period of activity the amplitude of the excursions is of the order of 30 microvolts and the frequency about 20 to 30 per second.

When the resting cat was stimulated by pinching a toe-pad of the hind limb contralateral to the active electrode, there followed a flexion reflex and usually turning of the head toward the stimulated foot. The change in cortical pattern was similar to that seen during voluntary movement; but, in addition, a large excursion usually occurred shortly after the sensory stimulus. In general, when the animal was thus aroused the pattern of cortical potentials was similar whether there was voluntary movement or not. This effect seems to correspond strikingly with that found in the human subject when sensory stimulation or concentration on a problem in mental arithmetic results in cessation of the typical 10-cycle (α) rhythm (Berger, 1930; Adrian, 1934).

In two of the three cats which we were able to observe without anesthesia, we found during sleep occasional groups of large waves (fig. 2c), larger than those recorded in the waking state. At other times when sleep was apparently less tranquil, judging by twitching of the vibrissae, there were only small rapid waves, as in the alert waking state.

In record d the cat seemed to be asleep. At the signal, the experimenter called; the only response observed was opening the eyes and moving the ears. The next day this cat was given a full dose of avertin. Record e was taken 4 minutes later. The cat was then moving vigorously. Two minutes later there was still some slight movement of the tail and legs (f). Fifteen minutes after the injection of avertin, the cat was completely under anesthesia (g). There were no spontaneous movements and no response to pinching the foot. Stimulating electrodes were then applied to the right sciatic nerve. Record h shows the response to stimulation with a tetanizing current (cf. section II).

In this series of records those under avertin anesthesia resemble those during rest and sleep, in that relatively large and slow waves predominate. Alertness and activity in the conscious animal and sensory stimulation during light anesthesia all show replacement of the slower waves by those of higher frequency.

II. Anesthetized Cats. When we come to observations under the anesthetics, we can generalize with more confidence than is possible from the small number of animals observed in the conscious state. We have made records from 12 cats under avertin, 16 under pentobarbital and 20 under ether, in which these anesthetics were used singly. In other cases the combined effects of two of these drugs were observed.

In comparing the actions of the three anesthetics employed, it is of interest to note the differences in the rapidity of their effects and of their elimination. Ether is the most rapid. Ordinarily, vapor from the ether bottle, at room temperature, undiluted with outside air, will induce a depth of anesthesia approaching stoppage of respiration in from 5 to 10 minutes. On withdrawal of ether, full return of reflexes and of brain-stem responses in the decerebrate cat is usually complete in less than 30 minutes (cf. Forbes and Miller, 1922). In the case of pentobarbital, the normal surgical dose produces its maximum effect in 8 to 10 minutes. The effect of the anesthetic wears off in from 10 to 20 hours. Avertin is the slowest of the three; the maximum effect of the surgical dose is reached in 15 to 20 minutes. The animal then remains under its influence for 24 hours or more.

A. Avertin anesthesia. The basal activity of the cortex under light avertin anesthesia shows excursions that are similar in type to those of the unanesthetized cat. The frequencies of the excursions can be grouped into those from 5 to 15 per second and of about 100 microvolts amplitude, and those between 20 and 30 per second. The latter form a continuous background and usually do not exceed 30 microvolts. In moderate depths the slower excursions appear in groups or "bursts," which sometimes appear at regular intervals.

Under deeper avertin anesthesia the excursions at 5 to 15 per second appear more definitely in groups. The frequencies of 20 to 30 per second are no longer visible, and in the pauses between groups the base-line may be nearly smooth. There is little or no reduction in the amplitude of the 5-to-15-per-second waves until the flexion reflex is very small. The groups become more infrequent in the deeper stages. Under still deeper anesthesia the waves disappear and the base-line becomes almost perfectly smooth, yet sensory stimulation still evokes a large electric response (fig. 3d).

In light anesthesia the responses elicited from the motor cortex by stimulation of the sciatic nerve may closely resemble those that accompany movement in the unanesthetized cat (fig. 3a). In some instances the response consists only of an increased amplitude in the rapid waves; usually there is also a decrease in amplitude of the slower waves. Slight negative or positive shifts of base-line have also been obtained. This means a change of potential, sustained throughout the duration of stimula-

tion, not necessarily accompanied by any change in the superimposed waves.

In moderate avertin anesthesia the responses of the motor cortex to sciatic stimulation are usually either abolished or so hidden in the basal pattern as to be indistinct. When the anesthesia is pushed to the level at which the interval between bursts is fairly quiet, then stimulation evokes a group of waves similar to one of the spontaneous bursts (fig. 3c). If the animal is more deeply anesthetized, the response to stimulation is composed of fewer waves. Finally, a state is reached in which the response is only one large excursion (see fig. 3d). The direction and form of the single excursion in the deep anesthesia are the same as those of the first big excursion of the burst characteristic of the lighter stage. This single excursion becomes reduced in amplitude as the flexion reflex disap-

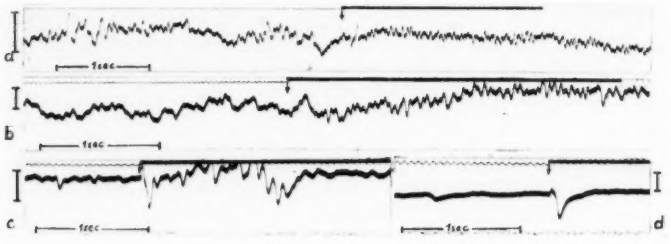

Fig. 3. Records from three animals showing typical motor cortex patterns and responses under various stages of avertin anesthesia. Duration of stimulation shown by heavy line above each record.

a, light anesthesia; b, moderate anesthesia; c, deep anesthesia; d, very deep anesthesia; respiration had just failed and had been restored. c and d, same animal. Sensitivity shown by line representing 100 microvolts beside each record.

pears and is finally lost at the death of the animal. The latency of this excursion varied in general from 40 to 60 msec. This variability may be due to the fact that in different experiments the active electrode was not placed on exactly the same position on the motor cortex.

B. Pentobarbital sodium. Under this anesthetic the basal pattern has excursions similar in duration or frequency to those found in the unanesthetized cat and under avertin. The typical pattern in moderate pentobarbital anesthesia is characterized by the frequent appearance of excursions resembling the fairly regular waves of about 6 per second sometimes found in the unanesthetized preparation. The amplitudes with pentobarbital, however, are much larger than those found without anesthesia, or with avertin, usually from 2 to 6 times as large. The predominant excursions are between 5 and 15 per second; while excursions of smaller amplitude, about 25 per second, are superimposed on them. Under light

pentobarbital, some cats have shown large slow waves of 1 to 3 per second and variable amplitude. The excursions between 5 and 15 per second range from 70 to 400 microvolts; while the faster waves of 25 per second are from 20 to 80 microvolts. The changes of pattern that occur with increasing depth of anesthesia consist in the appearance of the slower (now 5-to-10-per-second) waves in bursts, whereas the fast waves (25 per second) decrease in amplitude. Waves of the same duration and amplitude as the 5-to-10-per-second may appear as single excursions under a very deep stage of pentobarbital. Finally, with the loss of the flexion reflex, these potentials are also reduced and disappear on the death of the cat.

With the similarity of basal pattern produced under both avertin and pentobarbital, we find similar changes on stimulation of the sciatic nerve. Under light pentobarbital (fig. 4a), stimulation causes increase in ampli-

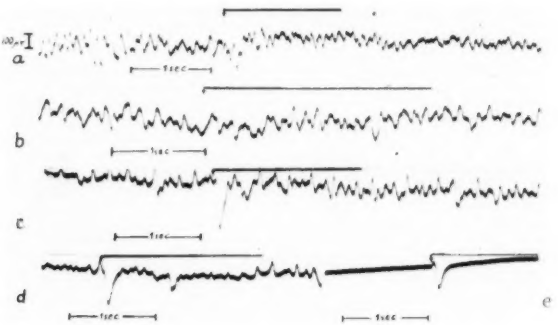

Fig. 4. Records of progressive stages of pentobarbital anesthesia, motor cortex, all from same animal. a, light; e, very deep. Stimulation shown as in previous figures. Sensitivity (same in all) shown by 100-microvolt line.

tude of the rapid waves, and sometimes a decrease in the slower waves. Sometimes a slight shift of the base-line has also been observed. With moderate depth of pentobarbital anesthesia, the 1-to-3-per-second rhythms are lost, and stimulation does not evoke any consistent and definite response (fig. 4b and fig. 7).

With deep pentobarbital, when the sciatic is stimulated, a large excursion appears in the record, with a latency of 40 to 60 msec. (fig. 4c). In this deeper stage the basal pattern shows little change beyond a slight decrease in continuity of the waves. In a still deeper stage, when the flexion reflex is nearly abolished, the basal pattern resembles that in very deep avertin, and during quiet intervals a large excursion similar to that in avertin follows sensory stimulation (fig. 4e).

C. Ether anesthesia. The patterns of the cortical potentials under ether

anesthesia do not resemble those of the resting unanesthetized cats in any respect, and they differ as strikingly from the patterns found with avertin and pentobarbital. The dominant frequency with this anesthetic is 30 to 40 per second, occasionally as rapid as 60 per second. These rapid waves are sometimes superimposed on large slow waves of 1 to 3 per second. The amplitude of the rapid waves does not exceed 40 microvolts nor do the slow excursions exceed 100 microvolts.

With increase in depth of anesthesia as determined by the flexion reflex, no marked change occurs in these basal patterns until the reflex is nearly abolished. At this depth the amplitude of both the slow and fast excursions decreases rapidly.

The changes produced by stimulation of the sciatic nerve vary with the depth of anesthesia. Under light or moderate ether anesthesia the re-

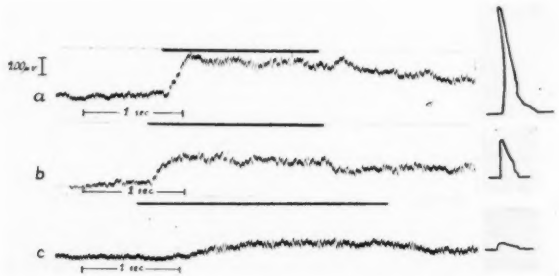

Fig. 5. Ether anesthesia, motor cortex. Three stages from light (a) to deep (c), showing great decline of base-line shift and small decline of rapid waves. At the right side of each record is a tracing of the simultaneously recorded reflex (knee flexion). Vertical line at left represents 200 microvolts.

sponse from the motor cortex was regularly characterized by a negative shift of the base-line (indicating negativity of the active lead) about 100 msec. after the beginning of stimulation and persisting as long as the stimuli continue (fig. 5a). Accompanying the shift is a marked increase in amplitude of the rapid excursions. In most cats the shift is preceded by a small positive excursion which has a latency of between 20 and 50 msec. The difficulty in assigning a definite latency to this response arises from the interference of the basal patterns with its beginning. In deeper stages of ether anesthesia, this shift is reduced until no response is obtained. Figure 5 shows three different stages of this anesthesia, and the drum records of the corresponding flexion reflexes. These shifts of base-line were a constant feature under ether and were much larger than the slight shifts sometimes found with the other drugs. In a large proportion of records from nearly every animal observed under ether, after 2 or 3

seconds of stimulation there appeared a series of regular slow waves (2 to 3 per second) outlasting stimulation. These waves seemed to have no effect on the fast waves superposed on them. They disappeared in the deeper stages of anesthesia.

That the striking differences between the patterns of activity under ether and under the other anesthetics are significantly consistent is shown not only by the regularity with which they were found in a large series of animals, but by the fact that in a single animal, if ether was withdrawn and avertin or pentobarbital administered, the pattern rapidly changed to that which was regularly found with that anesthetic, thus eliminating the possibility of ascribing the result to individual differences between animals.

The elimination of ether is so rapid that its withdrawal and the administration of pentobarbital constitutes a substitution of one anesthetic for the other, rather than a compounding, and thus affords a direct comparison of their effects. But when ether is given to an animal already under pentobarbital, the result is a compounding of anesthetic effects. We have regularly found that if ether was given when pentobarbital anesthesia was light the basal pattern changed to that typical of ether; but if ether was given when pentobarbital anesthesia was deep, the resulting pattern was more like that induced by an additional dose of pentobarbital.

The patterns obtained under pentobarbital and avertin were so similar that compounding their action gave results in which we did not find much difference from that of increasing the dosage of either one.

III. Localized Effects. Thus far we have described the spontaneous patterns of the motor cortex and the responses of this region to stimulation of the sciatic nerve. In a series of experiments, under ether, pentobarbital and avertin, we have placed electrodes on different parts of the cortex. We have found certain consistent regional differences, although, with relatively large electrodes, we have not determined the limits of the different fields nearly as definitely as Kornmüller has done in the rabbit. His results indicate a distinct localization of specific patterns on the cortex ("Feldeigenströme"). These bioelectric areas coincide strikingly with the architectonic fields. Kornmüller indicates a correlation between different frequencies and wave forms and the cell structure of the corresponding field. The cortical responses ("Feldactionsströme") to afferent stimulation are also localized according to architectonic fields. It should be noted that he found a decrease in regional differentiation under anesthesia. Travis and Dorsey (1932) recorded action potentials from the cortex of the rat, but they did not find any difference in pattern between different regions. The regions we studied were the sensorimotor cortex and areas adjacent thereto, the occipital cortex, and the auditory area of the temporal lobe. In a large proportion of our experiments we adopted for the active

leads the positions shown in figure 6. In what follows, these numbers will be used to designate the areas thus shown.

A. Avertin and pentobarbital. Under light and moderate stages with these anesthetics there was usually a difference in pattern between areas 1, 2 and 4, on the one hand, and 3, 5 and 6, on the other. Areas 5 and 6 differed from the others more strikingly than did area 3. The slower waves (5-to-15-per-second) are of much greater amplitude in areas 3, 5 and 6. The pattern derived from area 6 may be dominated by these slower waves (fig. 7f). In a few experiments there were observed some 1-to-2-per-second waves in regions 3, 5 and 6. Stimulation abolished them. The amplitude of the faster (15-to-25-per-second) waves is approximately the same in all regions. The response to stimulation in these different areas in light anesthesia is similar to the response from the motor cortex, described above. In the deeper stages the response is mostly indistinct. In very deep stages, when the flexion reflex is nearly abolished, the response consists of a single excursion (spike), as already noted in the case of the motor cortex.

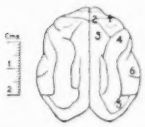

Fig. 6. Tracing of cat's cerebrum, showing sulci and positions of electrodes most commonly employed. *1* and *2* are on motor area; *3* is on sensory area for hind leg; *6* is on auditory area.

The most interesting difference found between different cortical areas was that which appeared in these large "spike" excursions which followed the onset of stimulation in the deep stages of both avertin and pentobarbital anesthesia. These large excursions, appearing clearly in response to stimulation, showed the following properties common to both anesthetics and to all areas observed. They appeared only when the anesthesia was approaching a depth at which the basal pattern of frequent waves was beginning to give way to one of inactivity characterized by a nearly smooth base-line. The latency of the major excursion was of the order of 40 to 80 msec. This excursion was usually preceded by a small excursion with a latency of about 10 msec., but the latter was recognizable only when the anesthesia was so deep as to afford a really smooth base-line. They were of transient duration, especially in the deepest stages of anesthesia, when there was a return to the initial base-line in 40 to 200 msec., and a continuance of complete inactivity throughout the period of stimulation.

The differences in these spikes as recorded from different areas are nearly the same with both avertin and pentobarbital. From areas 1 and 2 (motor cortex) the excursion was regularly positive (active lead positive with respect to ground lead on burnt cortex). From area 4, with one exception, the excursion was regularly negative. From area 3 (sensory) the responses were variable and complex, often appearing as diphasic deflections, usu-

ally with the initial excursion negative and the second excursion (usually the larger) positive. In areas 5 and 6 there was also some variability of response in the deep stages under consideration. With pentobarbital the majority of responses from these latter areas were large positive excursions; in a few cases they were smaller and negative. With avertin there was greater variability, diphasic excursions being common, with the positive phase (as a rule following the negative) usually the larger. A typical series of spike responses from the first five areas under deep avertin is shown in figure 8.

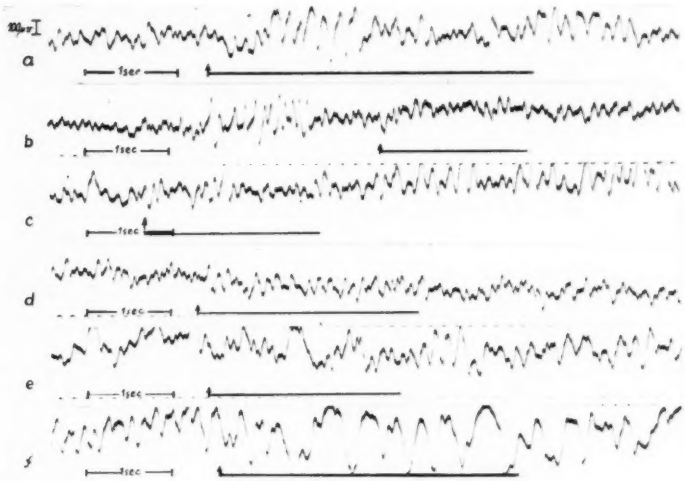

Fig. 7. Records from the six areas shown in figure 6 under medium pentobarbital. All records from the same animal within a few minutes. Sensitivity the same in all. Time of stimulation shown as in previous figures.

a, area 1; b, 2; c, 3; d, 4; e, 5; f, 6. Record e from area 3 in this animal resembled those of areas 1 and 2 more closely than area 5, differing in this respect from the majority of preparations.

In one experiment, a change of anesthetics was followed by reversal of the sign of the response obtained from the region of the lateral sulcus. The electrode was 4 or 5 mm. posterior to the usual position 3 (fig. 6). This area under deep pentobarbital showed somewhat variable responses consisting of one or two positive excursions. When the pentobarbital had largely worn off, the animal was put under deep avertin. When the base-line again became smooth the responses from leads 1, 2 and 4 were unchanged, but the response from the lateral sulcus showed a reversal, the dominant excursion being negative. These changes are shown in

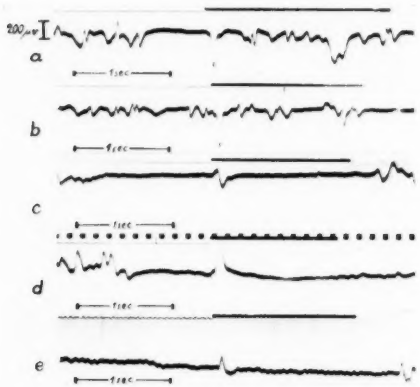

Fig. 8. Spike responses from different areas under deep avertin. a, area 1; b, 2; c, 3; d, 4; e, 5.

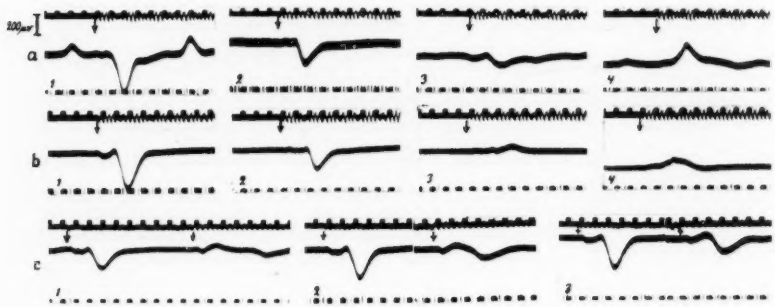

Fig. 9. Records at higher speed than previous figures, showing spike responses to tetanizing stimuli (a and b) and to single shocks (c), under very deep avertin and pentobarbital. Galvanometer without amplifier.

a, pentobarbital; b, avertin, administered about 3 hours after last dose of pentobarbital when anesthesia was already light. Electrodes in same positions throughout experiment. In a and b: 1, lead on area 2 (fig. 6); 2, on motor cortex posterior and lateral to area 2; 3, posterior to area 3; 4, area 4. c, avertin (soon after b), single shocks at various time intervals. Same lead as 1 in a and b. 1, make and break shocks, $\frac{1}{3}$-second interval (record continuous). 2, make and break shocks, $\frac{3}{4}$-second interval (part of interval omitted from record). 3, make and break shocks, 1.3-second interval.

Time shown by vertical lines at bottom of each record, marking 0.01 second. Arrows show beginning of stimulation in a and b, and single shocks in c.

figure 9, in which most of the records were taken at a higher speed than usual, for the comparison of latencies.

The exceptional case in which a positive spike was derived from lead 4 showed an interesting reversal. The excursion was positive under deep pentobarbital, but after the anesthetic had caused respiratory failure and the animal had been revived by artificial respiration the response was negative. This effect resembles those described by Neminski (1913).

In the deepest stages of avertin and pentobarbital anesthesia, when the base-line is smooth the response to continued tetanizing stimulation of the sciatic nerve is of brief duration. In some cases, of which examples are shown in figure 9, single shocks instead of tetanizing stimuli were applied to the sciatic nerve. The spike response in this case is of practically the same duration as in the case of sustained stimulation; from this it appears that only the first few stimuli evoke a visible response. In some cases single stimuli were applied at various intervals. With less than 1 second between stimuli the second response is reduced. Only after an interval of several seconds does the response attain its initial size.

In the case of the deepest stages of pentobarbital and avertin anesthesia, the smooth base-line facilitates a study of the latency of the large spike responses to sciatic stimulation. We have endeavored to compare the latencies from the different areas of the cortex. This major excursion as recorded from the motor cortex and the sensory area representing the hind leg appears with a latency of from 30 to 60 msec. When the active lead was placed on the regions more remote from the hind-leg area, namely, areas 5 and 6, the latency was usually appreciably longer, in the vicinity of 80 msec., although occasionally short latencies of only about 40 msec. were recorded from these areas also. Almost invariably, there is a small initial excursion with a latency of about 10 or 12 msec., which presumably represents the arrival of afferent impulses in the brain-stem, judging by other measurements of afferent impulses entering the brain (Forbes and Miller, 1922; Leese and Einarson, 1934). The polarity of this initial excursion varies according to the position of the electrodes. It probably represents the activity of nerve elements relatively remote from the electrodes.

B. Ether. We have described the spontaneous pattern and the response to sciatic stimulation from the motor cortex, under ether, and the contrast with the corresponding avertin and pentobarbital effects. With ether, the spontaneous pattern from other regions is similar to that of the motor cortex. The only difference we have found is that the occasional slow waves (1 to 3 per second) are apt to be larger in other regions than the motor cortex. But the responses to sciatic stimulation have certain differences in different regions. The negative shift of base-line, regularly present on the motor cortex, was sometimes obtained from regions 3 and 4. But these shifts were only slight and in one experiment the shift from region 4 was positive. We have already mentioned the appearance of rhythmic waves of about 3 per second which develop gradually within a

second or two of the beginning of the stimulation. This response could be obtained only under light ether anesthesia, and it is best developed on the region around the sulcus ansatus (fig. 10). From adjacent regions it could be recorded sometimes, but with less amplitude and less regular rhythm. In one experiment the amplitude of the largest wave was 100 microvolts and the entire effect was recorded for 16 seconds after stimulation ceased, when it was still continuing undiminished. The waves were not constant in amplitude but were alternately larger and smaller in a cycle of about 15 waves. Only once during this experiment did these waves appear in absence of stimulation. From the occipital cortex (area 5) an irregular response can be evoked by sciatic stimulation. From area 6 (auditory cortex) a response to sciatic stimulation was obtained. This region also shows responses to sharp acoustic stimuli.

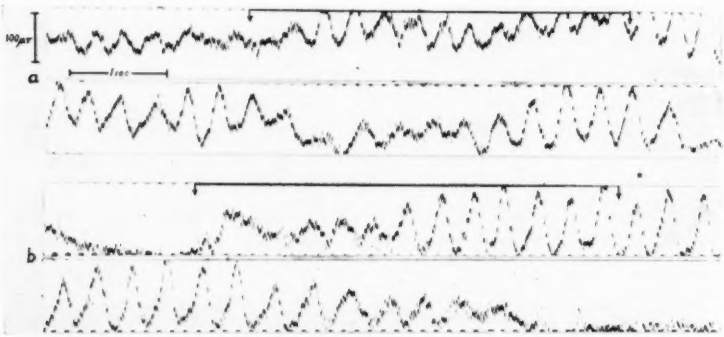

Fig. 10. Records of slow rhythmic response to stimulation under light ether. a, from area 3; b, from about 4 mm. posterior and lateral to a; both close to sulcus ansatus. In each case the lower record is continuous with the upper record. Duration of stimulation shown by heavy line between arrows.

It is evident from the above that with all three anesthetics employed we have found certain regional differences in the basal patterns and responses in the different parts of the cortex which we have studied, but these regional differences have in general been less marked than the differences between the patterns and responses which characterize ether and those of avertin and pentobarbital. Each of these drugs has given its characteristic pattern from all regions studied, and while the differences between pentobarbital and avertin have been less distinctive, the difference between these two and ether was striking, and consistently so from all parts of the cortex.

In some experiments the central end of the cut vagus was stimulated in an attempt to correlate susceptibility to the various anesthetics of the

reflex slowing of the heart and the flexion reflex. All three anesthetics abolished both reflexes in their deeper stages. There was some evidence suggesting that the vagus reflex was relatively more sensitive to ether than to the other anesthetics used, but the results on this point were too irregular to justify a definite conclusion.

IV. Asphyxia. In four animals we tried the effects of asphyxia on cerebral and reflex activity. In one of these, under moderate avertin anesthesia, asphyxia was induced by the rebreathing of expired air. In the other three, under moderate ether anesthesia, the mixture of ether vapor and air delivered to the tracheal cannula was replaced by nitrogen. In all cases the cortical waves disappeared at nearly the same time that spontaneous respiration ceased. At this stage sciatic stimulation produced a large excursion (active cortex usually positive) similar to those evoked in the deepest stages of avertin and pentobarbital (cf. fig. 9). At the same time the flexion reflex, instead of being depressed, as it was in the deep stages with all the anesthetics, was actually increased, usually greatly increased over its magnitude under the preëxisting degree of anesthesia. This striking increase in a spinal reflex during asphyxia is similar to that which we have often observed but not measured in decerebrate cats just before the onset of asphyxial convulsions following respiratory failure.

Discussion. Striking and consistent as our principal findings have been, it is difficult to evaluate their significance. The fact that pentobarbital greatly increases the amplitude of the slower waves suggests an analogy of its action to that of sleep. The work of Loomis, Harvey and Hobart (1935) and Gibbs, Davis and Lennox (1935) seems to show that in sleep there are often slower waves of an increased amplitude. The strikingly regular alpha rhythm of Berger, occurring when the subject is relaxed and relatively free from stimulation, suggests a tendency to synchronous discharge of larger groups of cells when the cortex is not subject to the stress of effort or stimulation. Perhaps the large waves under light pentobarbital can best be explained in like manner. The fact that ether regularly changes the picture in an opposite sense, by substituting for the slower rhythm a succession of smaller and much more rapid waves, suggests that ether interferes with the mechanism of synchronization. On the other hand, it has been noted that underlying this pattern of rapid waves under ether there is often evoked by stimulation a very regular series of large waves even slower than those which characterize the effect of pentobarbital. That all three drugs, especially in their deeper stages, greatly modify the basal pattern of cortical waves seems to indicate that they all act directly upon the cortex, and not merely upon afferent paths leading thereto.

The most important difference between the drugs is found in the deepest stages of anesthesia. It will be recalled that when ether anesthesia

becomes very deep the response to stimulation becomes smaller and finally practically disappears at about the time the flexion reflex is lost, even though at this stage the rapid waves, greatly reduced in amplitude, are still observable. In the case of both pentobarbital and avertin, the deepest stages are marked by almost complete quiet in the cerebral cortex, the record showing only infrequent waves to break the smooth base-line, but it is in this very stage in which the response to peripheral stimulation becomes most striking. The fact that the onset of stimulation is regularly followed by a large cortical response, appearing some 20 to 30 msec. after the first afferent impulse has entered the brain, seems to show that although the spontaneous activity of the cortex is largely, if not wholly, suppressed by these anesthetics, the sensory channel of approach is still not blocked as it is in the case of ether.

The recently reported experiments of Bremer (1936) agree perfectly with ours, in the comparison of basal patterns under ether and a barbiturate (dial) in its light and moderate stages. As with dial in his experiments, so with pentobarbital in ours, the effect of sensory stimulation disappeared at a moderate depth of anesthesia. Evidently the deepest stages in our experiments, when the effect of sensory stimulation reappeared as a spike potential, were much deeper than those employed in Bremer's observations. The stimuli, applied directly to the sciatic nerve in our experiments, were stronger than Bremer's stimuli, which consisted of pinching the foot.

It is especially noteworthy that in the case of deep avertin and pentobarbital the response to continued stimulation is almost as brief as the response to a single shock. Apparently something resembling fatigue (or "equilibration") such as has been described in the reflex arc (Gerard and Forbes, 1928) is present here in even greater degree. An alternative explanation may be an inhibitory effect resulting from impulses entering the brain via the more slowly conducting paths. We have no evidence in favor of this interpretation, but it cannot be excluded without further investigation.

SUMMARY

1. Action potentials in the cerebral cortex of the cat were recorded and the effects on them of anesthesia with ether, avertin and pentobarbital sodium observed. The cerebral response to stimulation of the sciatic nerve under these anesthetics was observed. At the same time the flexion reflex was recorded, as a measure of spinal activity.

Recording electrodes were applied, one to the intact cortex contralateral to the stimulated nerve, the other to the cauterized surface on the opposite hemisphere.

2. The patterns of cortical action potentials under light or moderate avertin or pentobarbital anesthesia differ comparatively little from those

in the unanesthetized animal. The chief difference is that under pentobarbital the waves are of greater amplitude. The frequency of these waves varies roughly from 5 to 20 per second. When the anesthesia is very deep, with these two drugs, the spontaneous waves become very infrequent. At this stage, sciatic stimulation evokes a large electric response from the cortex, appearing as a single excursion, or sometimes a diphasic potential, with a latency of 30 to 60 msec. and a duration not much greater.

Under ether the picture is entirely different, the basal pattern consisting of small, rapid waves of 30 to 40 per second, sometimes superimposed on very much slower waves (1 to 3 per second). Increasing the depth of ether anesthesia decreases the amplitude of the rapid waves without changing their frequency.

Stimulation of the sciatic nerve under light ether causes a negative shift of the base-line (active cortex negative), with increased amplitude of the rapid waves, and usually followed by the gradual onset of very regular waves of about 2 or 3 per second.

When ether anesthesia becomes deep, but before loss of the flexion reflex, these changes in response to stimulation become reduced till they practically disappear, although the rapid basal rhythm is still visible.

3. That a large cortical response to sciatic stimulation is obtained even after the cortex has become completely quiescent under avertin or pentobarbital, whereas no response to stimulation appears under deep ether, even though spontaneous waves are still visible, suggests that the two former drugs suppress activity in the cortex without blocking the sensory paths leading to it, whereas ether blocks these paths before cortical activity is wholly suspended.

4. By placing electrodes in different parts of the active cortex, regional differences in basal patterns and in responses to stimulation are found and described, but these differences are less striking than the differences between the patterns characteristic of the different drugs.

5. Asphyxia produced a picture similar to the deepest (moribund) stage of avertin and pentobarbital anesthesia, in that sciatic stimulation evoked a large electric response arising from a smooth base-line; but, whereas the flexion reflex was abolished by the anesthetics, it was increased in asphyxia.

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THE PASSAGE OF VISIBLE PARTICLES THROUGH THE WALLS OF BLOOD CAPILLARIES AND INTO THE LYMPH STREAM

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The passage of colloidal solutions, colloidal dyes and various proteins from the blood circulation to the lymphatics has been clearly demonstrated by numerous workers, but practically no data exist concerning the migration of particulate matter through the capillary endothelium, across the tissue spaces and into the lymphatics.

Some years ago, Krogh (1929) attempted to determine what he called the "absolute permeability" of the capillary, i.e., the size of the particles or molecules which could just pass through the capillary wall. Intravenously injected India ink whose particles were taken to be on the average about 200 mμ in diameter was held back in capillaries dilated by urethane while plasma was being filtered off. For this reason Krogh rejected any idea of the formation of fissures between the endothelial cells in dilated capillaries. Further experiments were carried out with soluble starch, the particles being approximately 5 mμ in diameter. These particles were found to pass through the endothelium when the capillaries were strongly dilated but were held back by normal capillaries. The two sets of experiments, incomplete as they were, left the absolute permeability of the capillaries of the frog at somewhat greater than 5 mμ but less than 200 mμ. The India ink injections cannot be considered to be conclusive because of the fact that India ink has a marked tendency to agglutinate in the circulation and because it adheres so readily to vessel walls that it leads to almost immediate obstruction.

OBSERVATIONS. *Graphite particles.* A suspension of graphite is more suitable for intravenous injections than is India ink because the graphite agglutinates slowly in the blood and adheres but little to the vessel walls. If an intravenous injection of a suspension, with particles of approximately 1μ in diameter, is made through the anterior abdominal vein of the frog, the material will be seen in the circulating blood of the web or tongue for a considerable period of time. Some of the graphite is removed by the liver, but a sufficient amount for observation remains in the circulation for many hours.

Phagocytosis of the graphite particles and the diapedesis of the graphite-laden phagocytes has been carefully studied in the tongue and in the web of the frog. It may take place within an hour after the injection, but more frequently in 2 to 4 hours. Extravascular graphite, however, is found before this time and careful high magnification examination has shown this graphite to be quite free. Electrical stimulation of the lingual nerves of the frog causes extravascular graphite to appear in the tongue in large amounts and in a short time. The exact manner in which the particles migrate through the wall is not known, but phagocytosis may be ruled out and the capillary endothelium is apparently physiologically intact. It is true that prolonged observation of the tongue invariably leads to abnormal conditions of the capillaries. But the same observations may be made upon the circulation in the web under conditions devoid of abnormality.

Calcite particles (*calcium carbonate*). A suspension of calcite, the particles of which were known to be from 1 to 2μ in diameter, was injected intravenously into frogs. It is easy to follow calcite in the circulation under the polarizing or dark field microscope because the particles are refractile and each one stands out as a bright point of light. The mesentery, moistened with oxygenated Ringer's solution, is one of the most favorable places for direct observation of the capillaries. The particles move along rapidly in the circulation, an occasional one adhering for a brief moment to the wall of the vessel only to become dislodged by the next passing corpuscles. Now and then a particle will adhere more tenaciously and will fail to become dislodged. Three times such a particle was observed to pass through the endothelium and to move slowly away from the capillary until there was the distance of the width of the capillary between it and the wall. The time of migration, once the particle had become firmly attached to the inner side of the endothelium, was from 5 to 15 minutes.

After carefully cauterizing the skin of the ankle in a frog anesthetized with urethane, a cannula was inserted and tied into one of the crural lymph sacs. One-half to 1 cc. of a calcite suspension was injected through the anterior abdominal vein. Eighteen minutes after the injection, the first drop of lymph which could be obtained contained 18 particles of calcite. A second drop of lymph obtained 38 minutes after the injection contained hundreds of particles.

Similar observations were made on both anesthetized and unanesthetized dogs whose lymphatics had been cannulated. The dog is distinctly less favorable for this type of experiment because of the rapidity with which foreign particles are removed from the circulation by the reticulo-endothelial system.

Experiment 1. Dog. Ankle lymphatic cannulated under novocaine; 15 cc. calcite suspension injected intravenously. Seven minutes after injection, during

which time the dog walked about in a perfectly normal manner, the lymph showed the presence of a few particles of calcite. The following samples of lymph, taken within the next hour, showed approximately the same numbers.

Experiment 2. Dog anesthetized with nembutal intraperitoneally. Cervical lymphatics and thoracic duct cannulated. 2:20 p.m., 15 cc. calcite suspension injected through jugular vein; 0.05 cc. specimens of lymph examined. 2:45 p.m., cervical lymph contained 2 particles of calcite; thoracic duct lymph, 40 to 50 particles. 2:53 p.m., 15 cc. calcite suspension injected intravenously. 3:10 p.m., cervical lymph contained 2 particles of calcite; thoracic duct, 2 particles. 3:45 p.m., 15 cc. calcite injected intravenously. 3:48 p.m., blood smear showed very little circulating calcite. 4:30 p.m., a few calcite particles on blood smear and in cervical and thoracic duct lymph.

From these experiments there can be no question of the actual movement of the calcite particles through the mammalian capillary endothelium into the lymph both in unanesthetized and in anesthetized animals.

Pneumococci. Experiments by one of us (Drinker, Enders, Shaffer and Leigh, 1935) demonstrated the passage of type III pneumococci, strain SV, virulent for rabbits, from the blood stream into the thoracic duct lymph. Rabbits injected intravenously with a large dose of this pneumococcus developed a bacteremia, and within an hour organisms could be cultivated from the thoracic duct lymph. The rapidity with which entrance into the lymph occurred appeared to be correlated with the size of the dose injected.

Since the thoracic duct lymph is largely obtained from the more permeable capillaries of the liver and intestine, the passage of the pneumococci into the cervical and leg lymph of the rabbit has been examined. Intravenously injected pneumococci appeared in the cervical lymph in about the same numbers and at the same time as in the thoracic duct lymph, and at the same time but in slightly fewer numbers in the leg lymph of the rabbit. Phagocytosis played no part in the emigration.

Erythrocytes. The presence of a few red blood cells in lymph seems to be a normal occurrence. In leg lymph these cells may become quite numerous after exercise and their appearance cannot be associated with any circulatory abnormality of the part. Emminghaus (1873) first observed that the number was considerably augmented by an increase in venous pressure, and this has been grossly confirmed by us (Drinker and Field, 1933) and by Weech, Goettsch and Reeves (1934) although no actual counts of the red blood cells in lymph obtained during increased venous pressure have been reported.

If the venous pressure in the leg of a dog is increased to 30 to 40 mm. Hg, the number of erythrocytes in the leg lymph will increase in a short time and will return to normal only when the venous pressure is lowered. This is illustrated in the following protocol. It is significant that when the venous pressure is increased a slightly larger flow of lymph is secured, but the total amount of protein in the lymph collected in four-

minute periods is not increased, indeed falls off toward the end of the period of increased venous pressure. This can mean but one thing, namely, that under increased capillary pressure red cells pass through capillary walls without permitting leakage of plasma.

Experiment. Anesthetized dog, nembutal intraperitoneally. Venous pressure recorded from a side branch of the saphenous vein in the groin. Lymph collected from a cannulated popliteal lymphatic by means of uniform, intermittent mechanical massage.

TIME	VENOUS PRESSURE	AMOUNT OF LYMPH IN 4 MINUTES	AMOUNT OF PROTEIN IN 4 MINUTES	RED BLOOD CELLS IN LYMPH
p.m.		*cc.*	*mgm.*	*per cu. mm.*
12:10	10	1.0	2.3	800
12:12	20			
12:25	20	1.0	2.5	400
12:40	20	1.0	2.4	0
12:55	20	1.0	2.3	1,000
1:00	30			
1:10	30	1.0	2.4	600
1:40	30	1.1	2.3	1,800
1:55	30	1.1	2.3	1,800
2:10	30	1.1	2.2	2,000
2:25	30	1.1	1.9	3,200
2:35	30	1.1	2.0	5,000
2:40	10			
2:55	10	1.0	1.8	4,000
3:10	10	0.8	1.3	3,200
3:25	10	0.7	1.3	1,600

Observations on the frog's leg are similar. If the femoral artery and sciatic nerve are freed and the rest of the leg lightly ligatured with embroidery cotton, a rise in venous and capillary pressure is inevitable. The diapedesis of a great many erythrocytes takes place in the web capillaries if the venous obstruction is moderately severe and prolonged. If, to a mild degree of venous obstruction, tetanic stimulation of the sciatic nerve is superimposed for varying periods of time, erythrocytes will almost immediately and in large numbers be visible outside of the capillary wall.

Microfilariae. The migration of microfilariae from the blood vessels to the lymphatics has been mentioned by one of us (Augustine and Drinker, 1935) in another connection, but will be referred to here briefly for the sake of completeness. The microfilariae (*Dirofilaria immitis*), found in dogs, are about 40μ in length and 5μ in width and are extremely active. The blood from an infected dog was given intravenously to a normal, anesthetized dog whose cervical and leg lymphatics and thoracic duct had been cannulated. Ten minutes after the transfusion, the blood of the recipient showed 178,000 microfilariae per cubic centimeter. At this

time 0.5 cc. of thoracic duct lymph contained 3 active microfilariae. Within the following hour the sediment of 48 cc. of thoracic duct lymph, collected during this period and centrifuged, showed 18,200 microfilariae. Twenty-five minutes after the transfusion a 0.5 cc. sample of leg lymph showed 1 organism, and in an hour a sample of 1.5 cc. contained 211 microfilariae. Samples of cervical lymph were negative for 3 hours but finally at the end of that period showed 9 organisms. Seven cubic centimeters of cerebrospinal fluid, collected at the end of the experiment, contained 464 microfilariae.

Discussion. The evidence presented here shows clearly that somehow or other non-motile particles in the blood, ranging in size from 1 to 2μ pass through the capillary walls into the tissue fluid and then through the walls of the lymph capillaries into the lymph stream. This, except in the case of the much larger microfilariae, is accomplished without motility and with too great a rapidity to be accounted for by phagocytosis. Nor is this movement of particulate matter confined solely to the more permeable capillaries of the liver and intestine from which the thoracic duct lymph is largely obtained, but it is a generalized phenomenon, the particles being found as readily in lymph from the cervical region and from the leg region where capillaries are considered to be relatively impermeable.

Through what part of the capillary endothelium and due to what force this passage of particulate matter is accomplished is still largely a matter of speculation. Florey (1926) has shown that in inflamed tissue a colloidal solution containing soluble starch passed directly through the cytoplasm of the endothelial cells rather than through the nuclei or through interendothelial stomata. The particles he was dealing with were probably about 5 mμ in size and are not representative of the larger particles with which we were concerned. Krogh (1929), in his early work on the absolute permeability of the capillary, abandoned the idea of the formation of any interendothelial stomata during dilatation because India ink failed to pass through the endothelium, but the whole idea of pores and spaces must be again reviewed in the light of the overwhelming evidence which has since accumulated as to the migration of particles of from 1 to 2μ in diameter. The same problem faces us in explaining the rapid absorption and appearance of such sizable particles in the lymph, for in order to gain access to the lymph stream they have, in some way, passed through a second physiologically intact barrier.

Recent work by Baron and Chambers (1936) upon the diapedesis of leucocytes and erythrocytes is of considerable interest in this connection. In the exposed mesentery of the frog they were able to watch both leucocytes and erythrocytes pass through capillary endothelium which had been irritated either mechanically or chemically. They observed that

the site of migration was always at an appreciable distance from the endothelial nuclear swelling, which suggested that the passage occurred between the endothelial cells. The attachment of the erythrocytes was not due to their adhesiveness but to their being caught in small spaces caused by retraction of the adjacent margins of the endothelial cells, and, once caught, they were forced through by the intracapillary pressure. The authors felt that the formation of temporary spaces between the endothelial cells did not cause any undue leakage of the capillary because, in the case of the leucocytes, the spaces through which they passed were formed while the latter lay directly on the endothelium. However, the openings through which the red cells passed were said to be present in the irritated capillary before the erythrocytes touched the walls, and although they almost immediately became plugged, it is difficult to see how there could be no loss of plasma. Baron and Chambers further found that the passive diapedesis of the erythrocytes was dependent on the capillary blood pressure, and that their rate of extrusion showed an increase which was dependent on the degree of irritation of the endothelium and the higher pressure coincidentally found in dilated capillaries.

Whether or not these same openings are present in normal capillaries is not known. Most of our experiments dealt with normal capillaries and normal capillary pressures as far as could be told, and under these circumstances the particulate matter passed easily through the capillary and lymphatic endothelium. It has been contended that the prompt passage of particles into lymphatics when material such as graphite is injected subcutaneously is due to wounding of lymph capillaries by the needle. To some degree this is undoubtedly true, but the evidence we have presented makes it clear that particles of many different sorts deposited in the tissue spaces from the blood capillaries enter lymphatics with the utmost readiness and under conditions precluding trauma to the lymph capillaries. It seems apparent that these passages of particles through intact capillary membranes resemble the passage of a globule of mercury through a gelatin film, a migration which leaves no trace of damage. One sees the same sort of thing on passing a needle through a bit of gelatin. Again no trace remains and at all times the gelatin has retained its integrity as a membrane.

SUMMARY

Experiments have been carried out which show that visible particles of many different sizes and physical characteristics pass through the uninjured walls of blood capillaries and frequently into lymphatics.

Graphite, with a particle size of 1μ, has been observed to leave blood capillaries in the tongue and web of the frog. Calcite, with a particle size of 1 to 2μ, behaved similarly in the mesenteric capillaries. The mate-

rial could easily be found in lymph from the foot of the frog and also in lymph from the foot of unanesthetized dogs.

Pneumococci injected intravenously in the rabbit appear rapidly in thoracic duct, cervical and foot lymph.

Erythrocytes readily become extravascular and are found in the lymph if the part is exercised or if the venous pressure is increased. No extra leakage of blood proteins accompanies this escape of red cells.

Microfilariae 40μ in length and 5μ in breadth readily leave blood capillaries and enter lymphatics. These organisms are large and highly motile. Their escape from blood capillaries is accomplished without injury to the vessels involved.

There is no evidence as to favored points of particle egress and the final nature of the passage is not known.

The authors wish to express their thanks to Miss Leonora E. Nash and Mr. Seymour M. Farber for certain of the observations included in this paper.

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PACEMAKERS OF HUMAN BRAIN WAVES IN NORMALS AND IN GENERAL PARETICS

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Recent work from several laboratories has shown that various groups of cells in the mammalian brain exhibit what appears to be "spontaneous" electrical activity, producing rhythmic waves of potential variations at characteristic frequencies. The most readily detectable of these rhythms, the alpha rhythm, also called the "Berger rhythm," has been shown (Adrian and Yamagiwa, 1935) to arise in a localized region of the occipital cortex. In man these occipital potential fluctuations of from 50 to 100 microvolts may be obtained, with a suitable recording system, directly through the skull merely by placing pad electrodes on the head, one preferably on the occiput and the other on a region anterior to the vertex. For recent reviews of the literature dealing with electrical brain waves, *cf.* Kornmüller, 1935; Jasper, 1936a.

The alpha waves from the occipital cortex of a resting human subject have been shown to occur at a frequency of about 9 or 10 per second. The rhythm is remarkably constant in a given individual and seems to be characteristic of activity of the otherwise "resting" cortical cells, since pattern vision usually inhibits it, probably due to a breaking up of the synchronized beating of the cells when they are thrown into specific patterns of activity by light (Adrian and Matthews, 1934). The rhythm is also inhibited during emotional disturbances. For a consideration of the rhythms during sleep and hypnosis, *cf.* Loomis, Harvey and Hobart, 1935a, b; 1936.

It is reasonable to suppose that the alpha rhythm from the occipital cortex may be due to the activity of a "relaxation oscillator" system[1]

[1] Relaxation oscillations, in contrast to those produced by reactions between elasticity and inertia, for example, are rhythms set up from any system in which a continuous source of energy stores up a potential which at some critical value discharges itself and in so doing tends to channel its pathway, thus releasing the charged store. A neon lamp with a parallel condenser in series with a resistance and a continuous source of E.M.F. flashes rhythmically whenever the P.D. across the condenser reaches a critical value. Many hydraulic examples exist (Fessard, 1931; Hill, 1933; cf. also Hoagland, 1935). Hill (loc. cit.) says "This type of oscillator (sometimes referred to as a relaxation oscillator) is the one with which alone we are concerned in physiology." Loeb (1900) seems to have been the first to apply this idea to brain physiology.

resulting from continuous, *entirely non-rhythmic*, metabolic events going on in the cortical cells. The frequency of the rhythm, therefore, might be expected to be directly proportional to the speed of these local metabolic events (*cf.* Hoagland, 1935). To test this hypothesis I have examined the alpha rhythm as a function of temperature in ten subjects who were given hyperpyrexia treatments at the Worcester State Hospital. I wish to thank Miss Alice Sheahan, technician in the Physical Therapy Department, for her kind coöperation in the experiments, and also Dr. F. H. Sleeper, Assistant Superintendent of the Hospital, for an independent analysis of the clinical records of the general paretic (tertiary syphilitic) patients.

If the rhythms were of the nature of relaxation oscillations their frequencies should be directly proportional to the velocity of certain underlying chemical events going on in the cortical cells and the Arrhenius equation should fit the data. This equation may be conveniently used in the form

$$\text{Frequency} = kv = ae^{-\mu/RT}$$

where v is the velocity of the underlying chemical mechanisms, e is the base of natural logarithms, R the gas constant, T the absolute temperature, k and a are constants, and μ the critical thermal increment, or temperature characteristic, in calories per gram mol of activating energy of the pacemaking step, i.e., the slowest process, in the catenary chain of the reacting system (*cf.* later "discussion" section, also Hoagland, 1935). Taking logarithms on both sides of this equation we get

$$\log \text{frequency} = c - \frac{\mu}{2.3\,RT}$$

where c is a constant equal to $\log a$. If the equation fits, a plot of log frequency against 1/T should yield a straight line of negative slope from which μ is readily calculated.

The subject was placed on a bed, thoroughly wrapped to prevent heat loss, and his temperature elevated by passing high frequency alternating currents through his body. Rectal temperatures were taken every fifteen minutes with a clinical thermometer during the one and a half to two hours that were necessary to elevate the temperature. Immediately after recording each temperature in the course of the procedure, electrodes were put on the subject's head, the high frequency current was turned off, the subject was blindfolded, and one or two minutes *later* the alpha rhythm was recorded continuously for some fifty seconds by means of a Garceau recording system consisting of an amplifier and ink-writing undulator recording on paper tape.

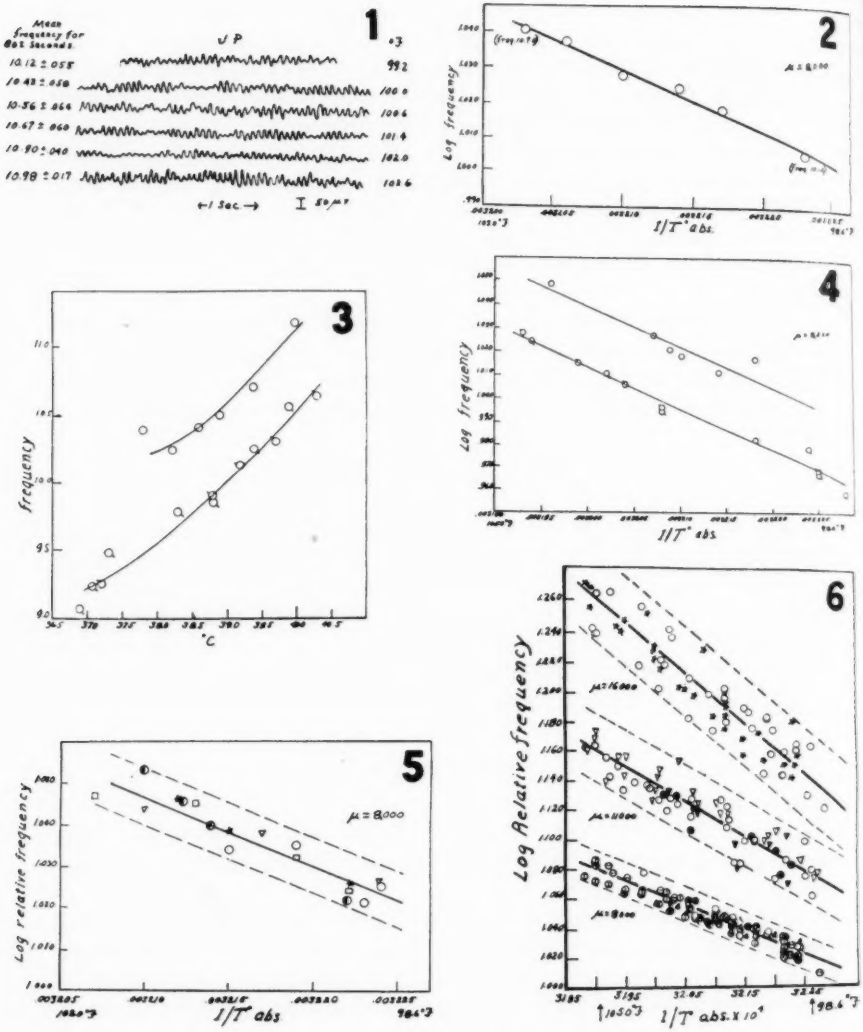

Fig. 1. Sample alpha cycles from a normal subject for temperature changes between 99.2 and 102.6°F. Mean frequencies for each temperature for 80 seconds of recording (approximately 800 cycles) with probable errors are shown at left. All brain wave reproductions in this figure and in figures 10, 11 and 12 are tracings.

Fig. 2. Arrhenius equation plot of the experimental data tabulated in figure 1.

Fig. 3. Direct plot of °C. vs. frequency of alpha cycles for three experiments with the least affected of the six general paretics. A slight exponential curvature is ap-

Of the ten subjects studied, three were normal controls, one was a mentally normal multiple sclerosis out-patient who came to the hospital weekly for diathermy treatments, and six were general paretics in various stages of the disease. Members of this last group were each given daily diathermy treatments for a two week period with temperatures elevated each time to 105.0°F. In this way it was possible readily to obtain numerous experiments on one individual with six or seven points per experiment for ascending temperatures and with additional points for descending temperatures in some of the tests. The multiple sclerosis patient was studied once a week over a five week period. His temperature was not elevated above 102.0°F. Three experiments were performed on each of two of the normals and one experiment only on the third normal. Temperatures of the normals were not elevated above 103.0°F. In all, some 230 records of mean alpha rhythms have thus been obtained from ten subjects as a function of temperature, and have been used as points in the plots that follow. Since each computed record averages in duration 50± seconds and since the mean frequency may be taken as roughly equal to 10 per second, there are thus seen to be involved in this study approximately 500 waves per point, or a total of 115,000 waves.

Figure 1 shows data from one experiment with one of the normal subjects. When the data of this experiment were plotted according to the Arrhenius equation, figure 2 was obtained, indicating an unusually good fit of this equation to the data. The critical thermal increment calculated from the slope of the line yields $\mu = 8{,}000$ calories.

Figure 3 shows a plot of data from three experiments done on different days with one of the least affected of the general paretics in which frequency is plotted directly against °C. It is interesting that two of these sets of data are described by the same curve, the third is definitely pitched at a higher overall frequency. This is what was found in many of the experi-

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parent. Each experiment is represented by a different symbol. In this, as in subsequent plots, approximately *500 alpha cycles* are averaged per point.

Fig. 4. Arrhenius equation plot of the data shown in figure 2. All three experiments yield $\mu = 8200$ calories, and linear relations are obtained. The different absolute frequencies of the upper and lower curve are characteristic of daily variations shown in many of the experiments on a single individual.

Fig. 5. Five weekly experiments on a mentally normal, multiple sclerosis outpatient. In this and in figure 6, the variability is shown by the vertical height between the broken parallel lines.

Fig. 6. Composite plot of all the data showing $\mu = 8000$ for three normals, the multiple sclerosis patient, and the two least affected paretics. $\mu = 11{,}000$ for two more advanced general paretics and $\mu = 16{,}000$ for the two apparently most advanced paretics. Solid points of the 11,000 band represent descending temperatures. The variability (width of bands) is seen to increase with the advancement of paresis.

ments in which some daily variation in the absolute position of the curves occurred.

Figure 4 shows an Arrhenius equation plot of the data of figure 3. It is clear that straight lines can be drawn through the data more satisfactorily than would be possible in the direct plots of figure 3. The value of μ for all three experiments is 8200 calories. It should be noted that the absolute frequency level on the grid is without effect on the slope of the lines which determine the value of μ. This constancy of slope indicates that while there may be day to day variations in the velocity of the overall chemical events in the cells producing the rhythm, the pacemaker, i.e., the slowest process in the assumed serial chain of reactions, remains the same (cf. Hoagland, 1935).

Figure 5 shows the results of five weekly experiments on the multiple sclerosis patient. In this figure, and in figure 6 the ordinates have been telescoped by multiplying by a suitable constant to bring the Arrhenius plots together on the grid, since we are not interested in the y intercept ($\log a$), but only in the slopes of the lines. The value of 8000 calories is of the same order of magnitude as that obtained from figures 2 and 4. In this figure different symbols represent different experiments.

The lower band of points of figure 6 shows a composite plot of the data from the three normal subjects, the multiple sclerosis patient, and two of the general paretics. From an independent analysis of the clinical records by Dr. F. H. Sleeper, the two general paretics included in this lower band were the least affected of the group of six paretics studied. The mean μ value for this group of subjects is 8000 calories. Different symbols represent different individuals. The middle band of points of figure 6 shows Arrhenius equation plots of data from two general paretics who have had the disease longer than the two yielding μ values of 8000 calories. The temperature characteristic is clearly 11,000 calories, suggesting a definite shift in the chemical pacemaker determining the oscillations with the more advanced state of the disease. The open triangles and circles represent ascending temperatures, for each of the two patients, the shaded triangles and circles descending temperatures. The uppermost band of points shows plots of data on two other advanced general paretics. One of these was the most advanced of the group; the other's early clinical history was incomplete and did not permit one to say that the stage of advancement was greater than in the 11,000 group, although her present picture is one of dilapidation greater than either of the patients in the 11,000 group. The mean μ value for both of these patients is 16,000 calories. The difference of slopes (μ values) for the three groups is clearly in evidence. The solid points of the $\mu = 11{,}000$ curve for descending temperatures indicate the *reversible*, specific nature of the temperature effect. The descending points fall within the band of "random" variability.

Figure 7 shows samples of the alpha rhythms at different temperatures in one of the patients yielding a μ value of 16,000 $\pm$ calories. The frequency increase with rising temperatures is, of course, more in evidence here than in the normal case shown in figure 1 in which the μ value is only 8000 calories.

From figure 6 it may be seen that the variability of the frequencies appears to increase with advancement of the disease as judged from the

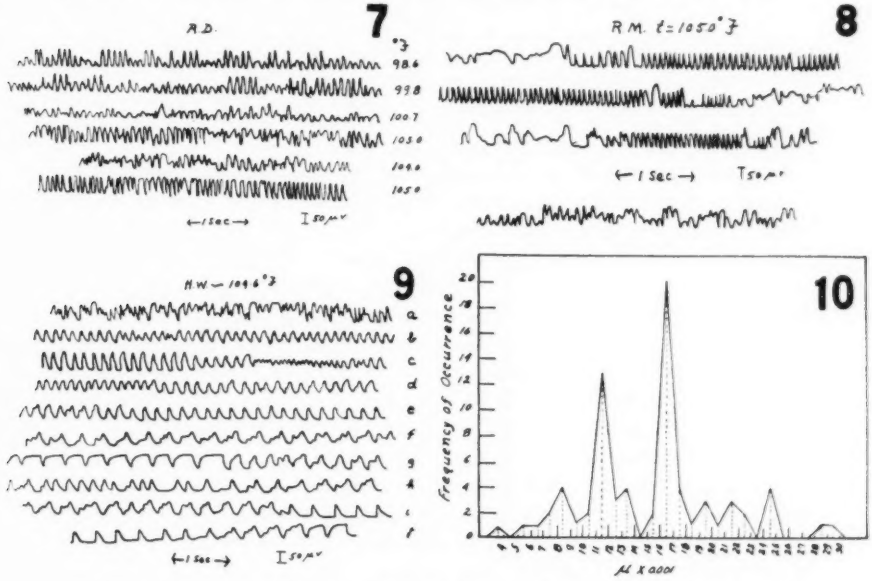

Fig. 7. Samples of alpha rhythms of an advanced general paretic (μ = 15,900) at different temperatures.

Fig. 8. For discussion see text.

Fig. 9. For discussion see text.

Fig. 10. Frequency distribution polygon for 74 determinations of μ values recorded in the literature by various workers for O_2 uptake and CO_2 production of plants and animals (whole organisms and tissues).

increasing width of the bands of points with increasing values of μ. This banded variability relation is similar to that usually encountered in Arrhenius equation plots of biological data. The latitude of variation is a constant percentage of the mean frequency and is *independent* of the temperature. Crozier and Stier (1924–25) have pointed out that the latitude of variation in typical temperature experiments with biological material is far outside the limits of possible errors of measurement and represents organic variation. From the lack of dependence of the variability on

temperature one may infer that the *physical* condition of the milieu in which the frequency of the phenomenon is determined is not significantly altered by temperature (loc. cit.). It is interesting that the curve yielding $\mu = 8000$ calories shows the smallest amount of variability.

Table 1 shows an analysis of variability of alpha cycles at normal body temperatures in terms of their probable errors. The frequencies in the first column are for six different individuals. Those in other columns are for the same individual on different days. It is clear that the probable errors of the alpha cycles increase in cases of the apparently more advanced paretics who yield higher μ values. Since 40 seconds of records (approximately 400 alpha cycles) are used to determine each frequency, and this number is the same in all cases, the probable errors are comparable measures of the variation in the different subjects.

At temperatures between 104 and 105°F. both of the advanced patients yielding $\mu = 16{,}000 \pm$ values sometimes showed an odd type of rhythm which I have not before seen described. On several occasions a surprisingly regular rhythm manifested itself, as may be seen in figure 8 from the patient R. M. ($\mu = 16{,}100$). At the time this rhythm occurred the patient, who usually gave clear alpha records at high temperatures, was restless and uncoöperative and the alpha rhythm was not determinable as may be seen at the beginning of the tracing. An example of her customary alpha record at 105°F. is shown in the lower strip of the figure. The highly regular rhythm appeared and faded out at intervals. Its frequency of $15\pm$ per second is higher than the $13\pm$ per second rhythm characteristic of this patient at 105°. The original rhythm was even more regular than could be reproduced by the free hand tracing in the figure. A sample of a similar regularity may be seen on the right of the last strip of figure 7 for the patient R. D. at 105° ($\mu = 15{,}800$).

Figure 9 shows a curious record obtained from a very dilapidated general paretic who gave such irregular alpha waves, and was so uncoöperative, that despite data obtained in five experiments no satisfactory μ calculations could be made. It is, however, worth noting that Arrhenius equation plots suggested a value of μ that would have been nearer to 16,000 than to 8,000 or 11,000, but the variability was very great. The strip marked *a* shows a fairly typical record at 104.6°F. The succeeding strips show the development of a sequence of curiously regular waves. The patient was awake and resting quietly and showed no unusual psychic symptoms. This sequence of waves developed in 2 out of 5 experiments when the temperature approximated 105°. Similar effects were not observed with any of the other patients. Ten to 20 seconds of records between *c* and *d*, *e* and *f*, and *f* and *g* have been excluded, otherwise the records are continuous. The long waves, 2 or 3 a second, especially apparent in *g* seem to be made up of the fusion of smaller waves as may best be seen in *j*.

Here single waves at 3 per second become transformed each into a catacrotic complex of 3 smaller waves (9 per second). These triple waves ultimately fuse into 1 long wave. Strip *g* shows such long waves breaking up spon-

TABLE 1

Frequencies with probable errors for 40 seconds recording of alpha rhythms at normal body temperatures

6 NORMAL SUBJECTS $\mu = 8000\pm$ FOR 3 TESTED NORMALS	O. P., A MULTIPLE SCLEROSIS PATIENT ($\mu = 8100$)	F. A., A G.P. ($\mu = 8200$)	F. C., A G.P. ($\mu = 7900$)	E. M., A G.P. ($\mu = 10,800$)	D. A., A G.P. ($\mu = 10,900$)	R. D., A G.P. ($\mu = 15,900$)	R. M., A G.P. ($\mu = 16,100$)
10.15 ±0.027	9.80 ±0.065	9.07 ±0.088	10.45 ±0.119	9.57 ±0.088	10.47 ±0.139	10.44 ±0.127	10.32 ±0.151
11.15 ±0.088	9.33 ±0.097	9.55 ±0.089	11.32 ±0.085	8.81 ±0.92	9.10 ±0.109	9.82 ±0.100	10.61 ±0.153
9.82 ±0.062	9.67 ±0.091	9.26 ±0.062	11.03 ±0.068	9.57 ±0.105	9.80 ±0.131	9.09 ±0.137	9.56 ±0.141
10.55 ±0.081	10.50 ±0.082	10.42 ±0.109		9.80 ±0.090	10.27 ±0.114	8.57 ±0.133	9.73 ±0.180
9.32 ±0.071				10.11 ±0.165	10.05 ±0.120	9.71 ±0.116	8.48 ±0.132
10.03 ±0.011					10.02 ±0.063	10.02 ±0.088	9.75 ±0.144
						9.05 ±0.163	10.32 ±0.134
Means 10.17 ±0.057	9.82 ±0.082	9.57 ±0.087	10.93 ±0.091	9.57 ±0.108	9.95 ±0.112	9.53 ±0.124	9.82 ±0.144

Mean probable errors for 40 seconds of records of alpha rhythms corresponding to different values of the temperature characteristic (normal body temperature)

$\mu = 8000\pm$	$\mu = 11,000\pm$	$\mu = 16,000\pm$
±0.080 16 experiments on 9 individuals P.E. is of mean frequency for approximately 4500 one second intervals	±0.108 11 experiments on 2 individuals P.E. is of mean frequency for approximately 3100 one second intervals	±0.134 14 experiments on 2 individuals P.E. is of mean frequency for approximately 3900 one second intervals

taneously into smaller, apparently component waves. The records, therefore, tend to support the point of view (Adrian and Matthews, 1934) that brain waves consist of synchronized potentials from many active units or

groups of units rather than slow potential changes of a mass of tissue as a whole. Mr. Shurrager (unpublished) in my laboratory has been able to isolate some interesting "spontaneous" electrical rhythms from the completely isolated olfactory lobes of the catfish. These waves appear at intervals to go through cycles of building and decline which closely resemble those shown in figure 9 *j* and *g*. The type of recording system used does not, however, permit of a detailed analysis of wave form.

Discussion. It is, of course, necessary to obtain data on more patients before clinical generalizations can safely be made about μ values for general paretics.

Jasper (1936b) has confirmed the finding of 8000 calories, earlier reported (Hoagland, 1936a) for the normal critical thermal increment of alpha cycles. He obtained values of 7000 to 8000 calories in normals and petit mal cases. (For a reply to criticisms raised by Jasper to my preliminary report, cf. Hoagland, 1936b.)

The fact that the Arrhenius equation describes the alpha cycle frequency as a function of temperature is not in itself especially interesting since so many physical and chemical processes follow equations of this type as functions of temperature. The μ values themselves, however, are significant since they coincide with the three major peaks in Crozier's (1925–26) multimodal frequency distribution polygon for 360μ values of different physiological processes. The peaks, in order of their prominence, occur at 16,000 (55 cases), 11,000 (43 cases), and 8000 (31 cases). Subsequent investigations have accentuated rather than diminished these peaks.

The above three values have been most frequently found to be associated with O_2 uptake and CO_2 production of cells. With the aid of a valuable compilation of μ values kindly sent me by Dr. T. Cunliffe Barnes, Mr. Zarrow (unpublished) has recently recorded the results of some 74 investigations from the literature in which μ values have been obtained for the gaseous metabolism of plant and animal tissues. Thirty-seven, or 50 per cent, of these publications have yielded μs falling within 8000–9000; 11,000–12,000; or 16,000–17,000 groups; i.e., 20 yield values between 16,000 and 17,000, 13 yield values between 11,000 and 12,000 and 4 yield values between 8,000 and 9,000. If the physiological μ values occurring in the range 2,000–30,000 calories varied at random, only approximately 10 per cent, instead of 50 per cent, should fall within the 8,000, 11,000 and 16,000 groups. Figure 10 shows the frequency distribution polygon for these respiratory μ values.

The repeated recurrence of specific μ values in respiratory experiments has been interpreted (Crozier, 1924–25) as representing energies of activation of corresponding slow steps in the series of essentially irreversible catalysed chemical events involved in cell respiration. The measured velocity of such a serial chain, as determined by direct gas measurements or by

indirect measurements such as the frequency determinations in these experiments, would of necessity depend upon the slowest process in the serial chain, in much the same manner as the slowest member of an assembly bench in an industrial plant acts as the pacemaker for the output of the product.

The soundness of this interpretation of μ values is further substantiated by the fact that it has been possible in certain cases to shift experimentally from one of these three values to another. For example, the rate of O_2 consumption of frog skin as a function of temperature at varying O_2 tensions up to 150 mm. yields μ = 11,300 calories. At tensions 350 mm. and above the μ value is 16,000 calories (cf. Crozier and Stier, 1925–26 data of Pütter). The μ for frequency of heart beat of *Limax* is normally 11,200 calories. Twenty-four hours after the ingestion of dextrose the value is 16,200, later returning to 11,200 (loc. cit.). The pharyngeal breathing movements of frogs yield normally a μ value of 8,250 calories. A week after destruction of the forebrain the μ is 11,000 (loc. cit.). It is interesting in this connection that Adrian and Buytendijk (1931) have shown that the completely isolated brain stem of the goldfish shows rhythmic fluctuations of potentials at frequencies corresponding to the normal opercular breathing rhythm. These observations have been confirmed by Mr. Shurrager (unpublished) using both goldfish and catfish.[2] The μ for normal opercular breathing in the goldfish is 16,500. After prolonged exposure to warm water the μ value becomes abruptly 8,300 (Crozier and Stier, 1925–26). What apparently happens in all these cases is a shift in the relative orders of magnitude of velocity constants involved in the cell respiration so as to make another step in the chain the slow pacemaker step. This interpretation is further borne out by the fact that sharp "breaks" often occur in Arrhenius equation plots for physiological data such that different and distinct μ values are found for the same process on either side of a critical temperature. This has been interpreted as resulting from the fact that the speed of the individual steps in the chemical chain is differently affected by temperature, thus shifting the ratios of the velocity constants so that for part of the range one is the slow pacemaker step with respect to the rest and for another part another reaction is slow (cf. Crozier, 1924–25; Hoagland, 1935). Shifts from one of the three values, 16,000, 11,000, 8000, to another of the three are not uncommon.

It is interesting that a value of 16,000 calories has been found repeatedly to be involved in the oxidation of Fe^{++} to Fe^{+++} (cf. Crozier, 1924–25) suggesting that an iron catalysed reaction may be the pacemaker where this value occurs. Values of 11,000 calories have been found frequently to be associated with hydroxyl ion activation (loc. cit.) implying the

[2] I have recently obtained similar electrical rhythms from the medulla in isolated brains of snakes, newts, and frogs. R. Gerard (unpublished) has also obtained electrical waves from isolated frog brains.

possibility of a dehydrogenation mechanism involved as pacemaker when this value occurs (cf. Moelwyn-Hughes, 1933).

From the results of these experiments it would, therefore, be reasonable to conclude that the alpha rhythm is directly determined by the local respiration of the cells of the occipital cortex. This respiration continually builds up electrical potentials which discharge as relaxation oscillations at critical potential values. The frequency would thus be proportional to the respiratory rate which in turn is set by the slowest link in the chain of respiratory events. In the general paretics the advancement of the spirochete infection of the nervous tissue appears to shift the pacemaker from a reaction requiring 8,000 calories to activate it to one requiring 11,000, and later still to one requiring 16,000 calories. This presumably results from shifts in the ratios of the velocity constants of the catenary processes involved in the cellular respiration. The decline of the frequency of alpha cycles with asphyxia produced by breathing nitrogen, as evidenced by the experiments of Gibbs, Davis and Lennox (1935), is in harmony with the interpretation of the dependence of the rhythm on oxidative processes within the cells.

I do not wish to imply from this study an over-simplification of the underlying mechanisms of brain waves. Many complex events are undoubtedly involved and numerous factors may, under some conditions, modify the frequency other than that of temperature. Under the conditions of the experiments the frequency at a given temperature in a resting subject is sufficiently constant to enable one to study the effect of modifications of temperature alone on the system. Temperature thus used as a tool indicates certain fundamental aspects of respiratory pacemaker dynamics regulating the rhythmicity.

SUMMARY

1. The frequency of alpha rhythms has been studied as a function of temperature in ten subjects whose temperatures were elevated by hyperpyrexia treatments. The Arrhenius equation was found to describe the data.

2. Three normals, one multiple sclerosis patient and two slightly affected general paretics in repeated experiments all gave a value of 8,000± calories for the critical thermal increment of their alpha rhythms. Two more affected general paretics gave 11,000± calories and the two apparently most affected general paretics gave 16,000± calories.

3. The variability of the data is analysed and discussed.

4. Certain curious and unusual brain wave records obtained thus far only with advanced general paretics between 104° and 105°F are described.

5. It is pointed out that the three critical thermal increments obtained are characteristic of those repeatedly found to be associated with cellular

respiration. The frequency of the alpha rhythm appears to be directly proportional to the speed of the underlying respiratory phenomena of the cortical cells which behave electrically as relaxation oscillators.

6. The advancement of the spirochete infection evidently shifts the pacemaker (slowest serial chemical process) involved in the respiration of cells of the occipital cortex from one normally requiring 8,000± calories for activation, to one requiring 11,000± calories, and later still to one requiring 16,000± calories.

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HYPERTENSION FROM CONSTRICTION OF THE ARTERIES OF DENERVATED KIDNEYS[1]

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Does the hypertension which results from limitation of circulation of the kidneys by constriction of the renal vessels (Pedersen (9); Bell and Pedersen (2); Goldblatt, Lynch, Hanzal and Summerville (4)) depend upon the nervous connections of the kidney? Menendez (7) in an unconvincing article answers the question in the affirmative. He maintains that, after denervation of the kidneys, renal vein constriction no longer results in hypertension. On the other hand Page (8) finds that the production of hypertension by constriction of the renal arteries is not affected by destruction of the extrinsic nerves of the renal pedicle. The following experiments, which were all started before the appearance of Page's article, are concerned with this same question, and the results are in complete agreement with those of Page.

METHOD. Essentially the experiments consisted in following the arterial blood pressure of male dogs in which both renal arteries were constricted and both kidneys denervated. Renal function was checked by means of the phenolsulphonephthalein test and by the blood non-protein nitrogen. The dogs were weighed once a week; in general there was little change in weight.

I. *Measurement of arterial blood pressure.* The method devised by Allen (1) has been used. A specially designed stethoscope bell is tied on the inner and anterior aspect of the hind leg just above the ankle joint. The cuff of a sphygmomanometer is wound around the limb so that the lower edge covers the stethoscope bell. The systolic and diastolic pressures are then determined by the auscultatory method. In my experiments the pressures were determined on the right hind leg with the dog lying comfortably on his side after a preliminary rest period of several minutes. Four readings were taken and averaged. Determinations were made at least twice a week, and were carried out for at least a month before the operative procedure in order to establish the normal level of blood pressure.

II. *Surgical procedure.* The operations were performed under aseptic

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

conditions, with the animal anesthetized by sodium pentobarbital administered intraperitoneally (40 mgm. per kgm. of body weight). An abdominal incision through the rectus muscle was made. This approach permitted the accomplishment of the surgical procedures described below on both kidneys in one operation.

Method of constriction. The technique employed to accomplish the constriction is patterned after a method which has been used for many years in Dr. F. C. Mann's laboratory (6) in connection with other blood vessels. A wire is laid alongside the artery parallel to its axis, and a ligature of braided surgical silk, size six, is looped three times about both artery and wire. The ligature is pulled snug so that the artery is completely constricted, tied, and then the wire is withdrawn, allowing the vessel to expand to an extent corresponding to the size of the wire. The degree of constriction may be varied by employing different sizes of wires; for dogs weighing about 20 kgm. a wire with a diameter of one millimeter was used.

Denervation. All tissue and nerves about the renal artery, vein, and ureter were meticulously removed. These structures were then scrubbed with dry gauze in order to clean them more thoroughly. Next they were painted generously with 5 per cent phenol in water. The kidney was then shelled out from the perirenal fat and peritoneum, leaving no connections whatsoever with the remainder of the animal except for the carefully cleaned artery, vein, and ureter. Constriction of the renal artery was then performed, if this procedure was to be done, and finally the kidney was replaced in its original position and the peritoneum sutured over it.

III. *Criteria of renal function. Phenolsulphonephthalein renal function test.* This test was performed as outlined in a previous publication (3). The phenolsulphonephthalein excretion for a period of one hour, after intravenous injection of the dye, was determined.

Blood non-protein nitrogen. The blood non-protein nitrogen was determined by the Koch-McMeekin micro Kjeldahl procedure (5), which is a direct Nesslerization method.

IV. *Postmortem examination.* All of the dogs except two have been killed, and autopsies have been performed on these animals. The period elapsing between the operation and the postmortem examination varied from 121 to 376 days. The eyes and slices of the kidney were preserved for histological examination. The heart and kidneys were weighed. In the case of the animals with denervated kidneys the renal arteries, renal veins, and ureters were saved and examined histologically for the presence of nerve fibers. I wish to express my appreciation to Prof. E. T. Bell of the Department of Pathology, University of Minnesota, for making the histological studies.

RESULTS. The experiments demonstrate that the destruction of the

extrinsic renal nerves does not prevent the development of hypertension when the renal arteries are constricted.

The 13 male dogs used may be divided into three groups. In six control animals the renal arteries were constricted; in three other control animals the kidneys were denervated; and in four animals both denervation and constriction were carried out.

In all cases the procedure or procedures were carried out in a single operation on both kidneys.

TABLE 1

Arterial blood pressure changes due to constriction of the arteries of kidneys with nerves intact and of denervated kidneys

DOG	SYSTOLIC PRESSURE			DIASTOLIC PRESSURE			INTERVAL BETWEEN OPERATION AND LAST BLOOD PRESSURE READING
	Average pressure before constriction	Final pressure (average of readings made in last 40 days)	Increase in pressure	Average pressure before constriction	Final pressure (average of readings made in last 40 days)	Increase in pressure	
	Nerves of kidneys intact						
	mm. Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*days*
1	131	168	37	61	98	37	180
2	128	158	30	58	87	29	337
3	149	187	38	60	112	52	184
4	127	159	32	48	83	35	376
5	127	170	43	66	109	43	183
6	154	192	38	68	127	59	408
	Kidneys denervated						
10	138	185	47	76	113	37	270
11	148	183	35	71	114	43	330
12	127	160	33	63	91	28	121
13	156	212	56	80	114	34	342

I. *Arterial blood pressure. Constriction alone.* In every one of these animals, in which the procedure was simple constriction of the renal arteries, an increase of both systolic and diastolic blood pressures occurred, with very little tendency to return to normal. The pressure started to rise very soon; in most cases it was elevated on the day following the operation. The results are given in table 1.

Denervation alone. Denervation does not affect the arterial blood pressure; for, in the three animals in which the kidneys were denervated, there was no significant alteration in either the systolic or diastolic pressure.

Denervation and constriction. All four of these animals, in which the arteries of the denervated kidneys were constricted, showed an increase in

TABLE 2

Heart weights and body weights of dogs with and without hypertension

	DOG	HEART WEIGHT	BODY WEIGHT	HEART WEIGHT EXPRESSED AS PERCENTAGE OF BODY WEIGHT	SYSTOLIC BLOOD PRESSURE
		grams	*kgm.*	*per cent*	*mm. Hg*
Hypertension. Constriction of renal arteries	1	235	25	0.94	168
	2	132	17	0.78	158
	3	262	31	0.85	187
	4	140	17	0.82	159
	5	215	24	0.90	170
Hypertension. Denervation and constriction	11	208	25	0.83	183
	12	134	17	0.79	160
	13	195	23	0.85	212
No hypertension. Denervation alone	7	139	18	0.77	150
	9	147	20	0.74	140
No hypertension		88	12	0.73	130
		230	30	0.77	
		121	16	0.76	130

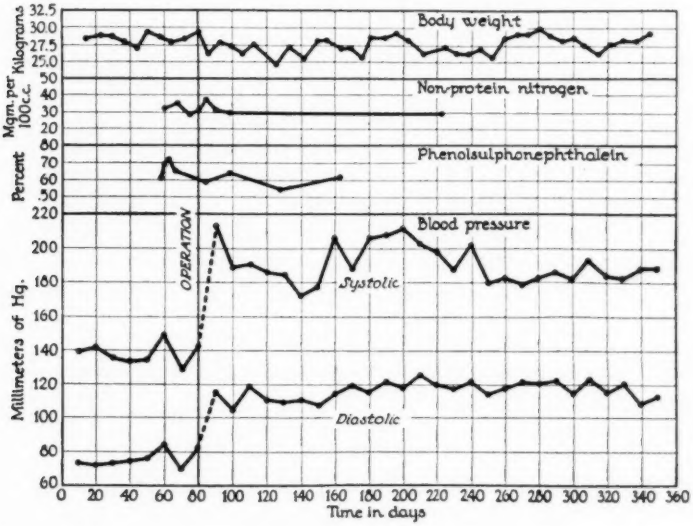

Fig. 1. Dog. 10. Denervation of the kidneys and constriction of the renal arteries. In the case of the blood pressure each point represents an average of the readings taken over a period of ten days.

arterial blood pressure. The results are also given in table 1, and the data for one of the animals are shown graphically in figure 1. These experiments lead one to the conclusion that the renal nerves are not involved in the hypertension resulting from constriction of the renal arteries.

II. *Completeness of denervation.* A most important consideration is the completeness of the denervation. The utmost care was taken in the destruction of the nerves. The histological examinations made by Dr. Bell of the structures of the renal pedicle did not reveal the presence of any nerves.

III. *Renal function.* Goldblatt, Lynch, Hanzal and Summerville (4) found that, unless both main renal arteries were suddenly almost completely constricted, the renal function was not materially reduced. The tests used in this research give no conclusive evidence of diminished function. The phenolsulphonephthalein excretion was not significantly low, nor was the non-protein nitrogen elevated. Apparently then, either kidney function is not impaired by the constriction, or the methods are not delicate enough to detect a change. One might reason teleologically that the hypertension is a compensatory mechanism for counterbalancing the increased resistance resulting from the constriction. As a consequence, unless the constriction be too severe, a normal renal blood flow and hence a normal renal function is maintained. Similarly clinical renal hypertension may be regarded as a compensatory measure for the increased resistance to blood flow due to pathological narrowing and occlusion of kidney vessels (2).

IV. *Pathological report. Kidneys.* The kidneys appeared normal grossly except for the development of collateral circulation through the vessels of the capsule in some cases. Doctor Bell could detect no histological abnormalities in any of the kidneys.

Nerves in renal pedicles. No nerve fibers could be detected histologically in the tissue surrounding either the renal arteries, renal veins, or ureters.

Eyes. No pathological changes in the blood vessels of the retinas were noted on histological examination.

Aortae. The aortae appeared normal grossly.

Heart. The heart weights merely suggest some hypertrophy, as may be seen from table 2.

SUMMARY

The arterial hypertension which results from constriction of the renal arteries is not dependent on the nervous connections of the kidney for its production.

Renal function, as indicated by the non-protein nitrogen and the phenolsulphonephthalein renal function test, is not significantly altered by bi-

lateral renal artery constriction of the degree employed in these experiments; nor are there any histological changes observable in the kidneys.

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WORK CAPACITY OF THE ADRENALECTOMIZED RAT TREATED WITH CORTIN

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Numerous clinical and experimental observations have established the fact that the administration of cortin tends to relieve muscular asthenia in adrenal insufficiency. The ability of the normal rat to continue work of the gastrocnemius muscle for five days or longer (2) is clearly distinguished from the performance of the adrenalectomized animal (1). In a study of partially adrenalectomized animals (3) it was shown that large amounts of cortical tissue are required to maintain normal work capacity while the medulla of the gland is completely dispensable for work under these experimental conditions (4). In the experiments to be described, the work capacity of the adrenalectomized rat treated with cortin was compared to that of animals whose adrenals were intact.

METHOD. Male rats having an age range of seventy to one hundred days and a weight range of 212 to 220 grams were used. The animals were grouped so that each individual experiment was conducted with from two to five rats, each matched within 3 grams for body weight and ten days for age. After a standard dosage of phenobarbital sodium, ether was administered for surgical anesthesia, and the adrenal bodies were removed with a single approach. A false operation in which the adrenal bodies were exposed but left intact was performed on one animal of each group.

Stimulation of the gastrocnemius muscle of each animal was begun within one hour after operation. The muscle was loaded with 100 grams and made to contract three times each second by direct faradic stimulation through silver needle electrodes. Each stimulus was of optimal intensity. The electrodes to the muscle of each animal were connected in series with the other electrodes in the stimulating current thus allowing the same shock to stimulate each animal. The muscular contractions were registered on automatic work recorders. The animals were enclosed in a cabinet in which the temperature was held at 28°C. At the beginning of stimulation and at each subsequent twelve hour period, appropriate amounts of phenobarbital sodium were administered, a solution of cortin diluted to 2 cc. was given subcutaneously in the neck, and 4 cc. of distilled water were injected subcutaneously in the posterior thoracic region. Stimulation

of the muscle was continued until the death of the animal or until one hundred twenty hours had elapsed. The apparatus and methods were modified after those described by Heron, Hales, and Ingle (2).

RESULTS. The initial rate of work for the animals which were subjected to sham operations averaged 6856 gram centimeters of work per minute with a range of 5600 to 8400 gram centimeters of work per minute. The

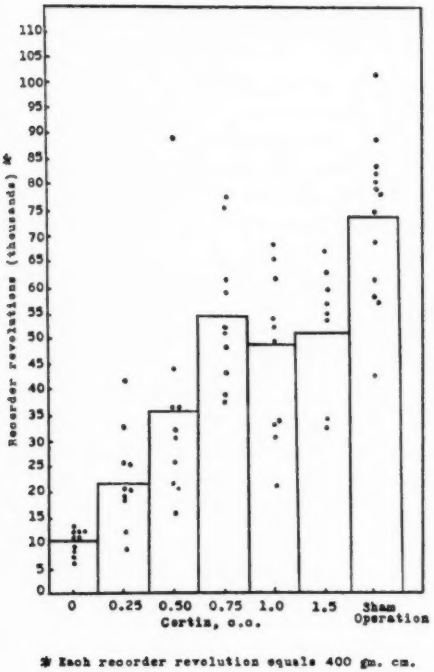

Fig. 1

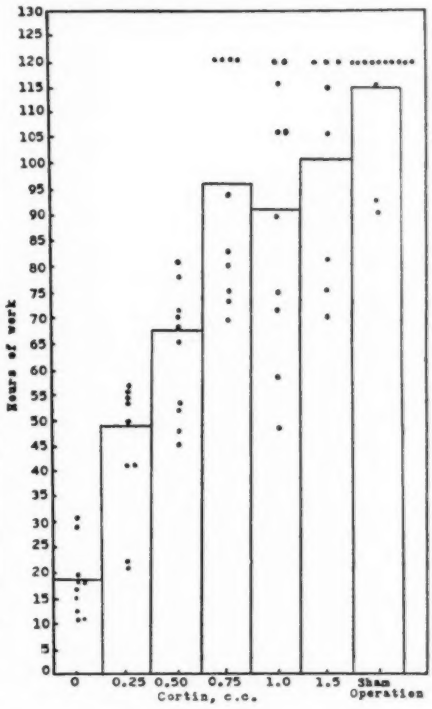

Fig. 2

Fig. 1. Relationship between amount of cortin and work.
Fig. 2. Relationship between amount of cortin and time of work.

average initial rate of work for the adrenalectomized rats was 6856 gram centimeters of work per minute, with a range of 4400 to 9600.

The times of survival are summarized in figure 1, and the records of total work are summarized in figure 2. The performance of the treated animals is clearly distinguished from that of the animals which did not receive treatment. Until the optimal amount of hormone was used the enhancement shows a direct relationship to the amount of cortin administered.

COMMENT. The sources of stress to the experimental animals are the operation, anesthesia, the injection of distilled water, and the performance of work. The trauma produced by adrenalectomy in the rat is slight and direct hemorrhage is negligible. A greater amount of trauma was intentionally produced in the sham operations. The amount of anesthetic agent administered and the amount of distilled water injected were kept constant in each animal. Each animal received the same stimulation and worked against the same load. The deficiency of work performance and times of survival on the part of the untreated adrenalectomized animals is clearly conditioned by the absence of the adrenal bodies. It has been demonstrated by the author (unpublished experiments) that the rapid collapse of the adrenalectomized animal under these experimental conditions cannot be duplicated by hypophysectomy, thyroparathyroidectomy, gonadectomy, thymectomy, nearly complete pancreatectomy, nephrectomy, extirpation of the entire gut, loss of 25 per cent of the total blood volume, or by lesions in any part of the hypothalamus.

The work performance of the adrenalectomized rat is slightly improved by the administration of sodium chloride, but the effect of cortin has not, up to the present, been duplicated by any form of therapy with sodium chloride, sodium bicarbonate, or dextrose. Administration of sodium chloride in addition to cortin definitely lowers the demands for this hormone by the working animal. The injection of epinephrine has a temporary enhancing effect on the height of muscular contraction in these animals just as it does in the normal "fatigued" animal, but the ultimate effect is invariably deleterious when measured by either work totals or time of survival.

The failure of the animal treated with cortin to duplicate the average performance of the animal subjected to a sham operation cannot be satisfactorily explained. One obvious difference in the two conditions is the intermittent injection of the hormone as compared to the continuous secretion of the hormone by the glands themselves. It is conceivable that an excess of cortin in the blood stream immediately after injection would have a deleterious effect and that a deficiency level might occur prior to the next injection. There are other possible explanations for the deficiency.

The administration of cortin to the previously untreated working adrenalectomized animal at the time of collapse is usually effective in bringing about a marked recovery in the height of muscular contraction within thirty to sixty minutes, which may be maintained for many hours. Spontaneous recovery never occurs and the administration of cortin is the only effective form of therapy. The importance of the loss of sodium, chlorides and bicarbonate, and of depletion of carbohydrate reserves in the body is well established but it is significant to note that marked recovery of work capacity and prolongation of survival time can be effected by administration of cortin alone without the replacement of these other substances.

During the past eighteen months the enhancing effect of cortin on the work performance of the adrenalectomized animal has been applied in the biologic assay of extraction of the adrenal cortex prepared in the biochemistry laboratory of The Mayo Foundation. The maintenance of muscular contractions for forty-eight hours in the adrenalectomized rat is certain proof of biologic activity of the extracts. The failure of extracts to prolong the time for work is an equally reliable proof of lack of biologic activity. Use of an apparatus which allows the stimulation of eight animals simultaneously provides a method for rapid assay, which is fully as sensitive to differences in the concentration of the hormone as is any other method. There is excellent agreement between the assay values assigned by this method and independent values assigned by assays by the Pfiffner urea method (5).

SUMMARY

Rats which were subjected to sham operations were compared to adrenalectomized rats, both with and without cortin treatment, for ability to withstand work and anesthesia. The animals were anesthetized with phenobarbital sodium, and the gastrocnemius muscle was stimulated to lift a 100 gram weight three times each second. Administration of cortin to the adrenalectomized animal enhanced its work capacity in proportion to the amount of cortin administered until an optimal dosage was reached. The average performance of the rats treated with cortin remained below that of those animals whose adrenal bodies were intact.

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THE EFFECT OF BREWER'S YEAST ON BLOOD PRODUCTION

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The most common methods employed in recent years for determining the values of various food materials in overcoming an experimentally produced anemia have been the method proposed by Whipple and his associates, and that developed by Mitchell, Hart, Steenbock and others. The most outstanding contributions made by the use of these two methods are the unique value of liver and the necessity of copper in the utilization of iron.

Whipple made dogs anemic by bleeding them at whatever interval was necessary to keep their hemoglobin at one-third its normal value. The value of various supplements in producing new hemoglobin was determined by measuring the amount of hemoglobin removed during a two-week period in excess of that produced by the basal diet. Mitchell, Hart and others made rats anemic by raising them on an iron-deficient diet.

The purpose of this paper is to report results obtained by a different method of approach. White rats raised from stock animals on a uniform diet were placed, as soon as weaned, on a basal diet made up as follows: casein (vitamin-free, Harris) 18 parts; Crisco, 2 parts; cod-liver oil (Mead's), 3 parts; bleached dextrin, 65 parts; salt mixture (1), 4 parts; and brewer's yeast in variable amounts. As will be noted, yeast constituted the only variant. The animals were segregated into groups of eight. One group was fed the basal diet with no yeast at all, another group the basal diet plus two parts yeast, another four parts, another eight and the fifth group sixteen parts of yeast. When the first group showed a definite cessation of growth, four of each group were sacrificed and the following data obtained: per cent of hemoglobin, number of red and white corpuscles, reticulocyte count, red cell volume, and the determination of the fragility of the erythrocytes. The remaining four of each group together with an adequate supply of food and water were then placed in a decompression chamber and the pressure gradually reduced until it was equivalent to that existing at an altitude of 20,000 to 25,000 feet (375–320 mm. Hg). After the animals had been subjected to this pressure for ten days they were removed and data similar to that mentioned above obtained. It has been

shown that a ten-day period is adequate for the establishment of an equilibrium.

Subjecting an animal to a rarefied atmosphere introduces at once a powerful stimulus to hematopoiesis and, under these circumstances, also a continuing stimulus. The ability of the animals to respond to this stimulus will be conditioned by the reserves of blood forming elements they have stored in their tissues and this, in turn, will be conditioned by the presence of these elements in their food. In the experiments to be reported herein, the only variant in the food is the yeast, so that any variation in the experimental data can be attributed to this one factor.

Yeast is not a simple compound, and, for this reason, difficulty may be encountered in assigning an effect to its proper cause. According to Parsons and Hickman (3), yeast is not only rich in the vitamin B-complex, but contains iron in both inorganic and organic form, copper, manganese and, in brewer's yeast, a trace of arsenic. It also contains the two proteins, zymocasein and cerevisine. The various constituents of yeast could not have supplemented the basal diet used in these experiments in respect to protein, iron or manganese. While no copper was added to the salt mixture used it is inconceivable that copper was not present in adequate amount in the materials used. Arsenic also was not added, but in view of the results obtained by Beard and Meyers (4), we are not inclined to attach much importance to it. From this survey, it should be evident that the chief factors contributed by yeast to the basal diet were the vitamins which it contained.

Results. Control data were obtained from twenty-three normal stock animals. An average hemoglobin figure is thus seen to be 111 per cent; the red cell count 7,670,000; the white cell count 17,850; the reticulocytes 2.5 per cent. The average hematocrit reading showed that practically 42 per cent of the blood volume was made up of red cells. The average red cell hemolyzed in a hypotonic saline solution of 0.46 and 0.48 per cent concentration. Similar data on experimental animals may be obtained from table 1.

Fifteen rats, as soon as they were weaned, were arranged in two groups and placed on the basal diet to which no yeast had been added. When their growth curves began to flatten out, it was found that group A had an average hemoglobin value of 93.6 per cent and a red cell count of 7.72 million, while group B had a hemoglobin value of 102 per cent and a red cell count of 4.822 million. The chief difference in the two groups lies not so much in their blood values as in their latent ability to develop red cells and hemoglobin when living in a rarefied atmosphere. Group A which was placed in the decompression chamber for a period of five days succeeded in raising its hemoglobin to an average value of 117 per cent. The red cell count, however, decreased to 7.03 million. One of the animals of this

group of five died while in the chamber. This particular rat was very anemic before being placed in the chamber and died undoubtedly because of an inability to transport oxygen sufficient for its requirements. Group B was maintained on the B deficient diet for a longer period than group A, so long, in fact, that two animals were having convulsions when the group was placed in the decompression chamber. Needless to say, these two did not survive. At the end of ten days, the four surviving animals had

TABLE 1

The number of erythrocytes and the amount of hemoglobin of rats on the basal diet and diets supplemented by various amounts of yeast before and after exposure to low barometric pressures

DIETARY SUPPLEMENT	BEFORE BEING IN CHAMBER		AFTER BEING IN CHAMBER		DAYS IN CHAMBER	NUMBER OF ANIMALS
	Per cent Hb	Red cells	Per cent Hb	Red cells		
		millions		*millions*		
Basal diet	93.6	7.720	117.0	7.030	5	5
Basal diet	102.0	4.822	150.0	9.083	10	10
Average	97.8	6.271	133.5	8.056		
2 per cent yeast	95.2	6.800	120.0	7.282	5	5
2 per cent yeast	94.6	7.286	135.2	5.318	10	5
2 per cent yeast	100.0	7.340	157.0	6.165	10	8
2 per cent yeast			138.5	6.508	13	4
Average	96.6	7.142	137.7	6.318		
4 per cent yeast	88.6	6.680	117.0	6.970	6	5
6 per cent yeast	93.6	7.560	126.4	6.680	5	5
8 per cent yeast	90.8	6.750	107.8	6.720	5	5
8 per cent yeast	103.6	7.052	151.2	6.870	12	5
8 per cent yeast			144.0	7.712	14	5
Average	97.2	6.901	134.3	7.101		
16 per cent yeast	104.0	9.150	156.0	11.900	10	8

an average hemoglobin value of 150 per cent and a red cell count of 9.083 million. The high values obtained by group B are believed to be the result of the anhydremia which develops as a terminal affair in B avitaminosis (6, 7).

A survey of the above experiment will reveal the fact that there are two considerations which deserve further discussion, namely, the effect of the length of the period in the chamber and secondly, the effect of the anhydremia which develops during the latter stages of B avitaminosis.

Drastich (2) showed that the greatest increase in the number of red cells of guinea pigs exposed to rarefied atmospheres occurred within eight days. Our own experience shows that after ten days there is very little if any variation in the number of red cells. An examination of the results obtained from the groups receiving 2 per cent and 8 per cent yeast illustrates this point (table 1).

Sure (7) showed that dogs suffering from a deficiency of the B complex experienced an increase of 13.7 per cent in hemoglobin as a result of anhydremia. The same has been shown to be true for young rats (6). The compensatory rise in hemoglobin and red cells when animals are placed in a rarefied atmosphere is liable to be obscured by the apparent increase in the same constituents because of anhydremia. The data in table 1 demonstrated this point by showing the highest concentration of hemoglobin and number of red corpuscles at the two extremes of vitamin B feeding.

The group of animals on the basal diet only showed a final red cell count averaging slightly over 8,000,000 and a hemoglobin content of 133.5 per cent. The only group that exceeded these values was that receiving 16 per cent yeast. A yeast supplement as low as 2 per cent is sufficient to protect the animals from vitamin B deficiency and its consequent blood concentration. The figures in table 1 show that there is a greater relative increase in hemoglobin than there is in the number of red corpuscles. Nevertheless, with the exception of the one group receiving the 4 per cent yeast supplement the number of red cells increased as the yeast increased. While it is true that the red cells tended to increase as the yeast increased, this must not be confused with the fact that the relative number of red cells (with the exception of the group receiving 16 per cent yeast) showed very little, if any, increase. As a matter of fact, two of the groups suffered a loss in red cells, while the remaining two groups experienced only a 3 or 4 per cent gain which can scarcely be considered significant. Perhaps a better statement of the situation would be to say that as the yeast increased there was a smaller diminution in red cells. There was far more room for improvement in hemoglobin than there was for an increase in red cells. It is obvious that the presence of yeast in the food intake of these animals provided some element, or elements, that were necessary in the fabrication chiefly of hemoglobin, and, to a less extent, of erythrocytes.

Many times early changes in the hematopoietic tissues are not manifested in the peripheral blood stream, but are capable of being observed in the bone marrow. For this reason, it was deemed advisable to make differential counts of the various cells found in the bone marrow of rats on a B deficient régime after their exposure in the decompression chamber. These animals were removed and bled to death. The serum obtained from the blood was used to dilute the bone marrow. Three animals from the group receiving the basal diet only were examined in this way. One animal from

the group receiving a supplement of 8 per cent yeast served as a control. The results are to be found in table 2. It will be observed that the control rat had 19.4 per cent normoblasts and 1.4 per cent megaloblasts in the bone marrow. In contrast to this, the normoblasts of the experimental animals ranged between 0.4 and 1.0 per cent and the megaloblasts from 1.2 to 2.8 per cent. These results are interpreted as indicating that the demand for an increased output of red cells by the experimental animals is being met on the part of the bone marrow by an increased production of megaloblasts, which, however, fail to progress to the stage of the normoblast.

TABLE 2

The differential bone marrow count of rats on the basal diet and on the basal diet supplemented with 8 per cent yeast after exposure to low barometric pressure

BONE MARROW CELLS	CONTROL RAT—8 PER CENT YEAST SUPPLEMENT	EXPERIMENTAL RATS—BASAL DIET ONLY		
		No. 2	No. 5	No. 6
Small lymphocyte	28.6	43.6	34.4	39.6
Polymorphonuclear	22.8	39.2	39.4	46.3
Staff cells	1.6	7.2	12.4	4.6
Eosinophils	5.2	2.0		0.4
Promyelocytes	9.4	0.8	2.4	1.8
Degenerated cells	6.4	2.8	3.2	3.0
Monocytes	1.0	2.0	3.6	1.8
Basophiles	1.4	0.4	0.4	
Normoblasts	19.4	0.4	0.4	1.0
Stem cells	2.4	0.4	1.0	0.4
Megaloblasts	1.4	1.2	2.8	1.2
Myelocytes	0.2			
Megakaryocytes	0.2			

The normal body has recourse to many different methods of effecting a compensation in the face of a rapidly or for that matter, a slowly developing crisis. When an oxygen deficit constitutes the crisis, the most commonly employed compensatory mechanism involves an increased respiratory and cardiac rate, contracture of the spleen, curtailment of activity and increased output of red cells and hemoglobin on the part of the bone marrow. There may be still further mechanisms whereby the blood may maintain a proper oxygen transport in the face of increased red cell destruction or decreased production. For example, the red cell in some manner may become more resistant to disintegration and thereby function for a longer period of time than would ordinarily be the case. Because of such considerations, the foregoing data were supplemented by the following: white cell and reticulocyte counts, red cell volume and fragility determinations.

Consideration of table 3 will show that there is a definite increase in the number of reticulocytes which in itself does not indicate an absolute increase in red cell production. The white cells suffered very little if any change. The volume of red cells in the plasma has increased considerably. This is true of all the groups and, since there was not a general increase in the number of erythrocytes, demonstrates clearly an increase in the average size of the single red cell. According to Witts the presence in the peripheral blood of nucleated red blood cells is a sign only of hyperplasia of the bone marrow and is not evidence of increased blood formation unless they are accompanied by an increased number of reticulocytes. An increase in the per cent of reticulocytes may not represent an absolute increase in red cells, but simply a relative increase in the more immature forms (8). There is also a definite increase in the ability of the red cell to withstand the effect of hypotonic solutions. Normally the red cell hemolyzes in a 0.42 saline

TABLE 3

The number of leucocytes and reticulocytes, the volume and fragility of the red cells of the blood of rats before and after exposure to rarefied atmospheres

DIETARY SUPPLEMENT	BEFORE PLACED IN CHAMBER				AFTER BEING IN CHAMBER			
	White blood cells	Reticulocytes	Hematophiles	Fragility	White blood cells	Reticulocytes	Hematophiles	Fragility
Basal only			47.7	0.37				
2 per cent yeast	9,425	2.55	39.5	0.45	16,000	11.25	55.5	0.39
2 per cent yeast					12,100	11.50	51.8	0.37
8 per cent yeast					10,450	8.00	56.5	0.37
16 per cent yeast	16,675	2.35	40.0	0.42	10,200	5.05	57.0	0.40

solution. The red cells of rats exposed to rarefied atmospheres do not hemolyze until the concentration of the saline has been reduced to 0.38 per cent. The reason for this may be due to the failure of the red cell to be exposed to the influence of the spleen which under these experimental conditions must be in a permanently contracted state.

DISCUSSION. So much has been written on the subject of anemia, especially nutritional anemia that it does not seem necessary to cover the literature in a brief article such as this. The purposes of this report seem to be best served by discussing the possible bearing of yeast upon erythrogenesis. There is no doubt but that factors contained in yeast exert a beneficial effect in anemia. For example, Wills has shown that it is definitely more effective in pernicious anemia of the tropics than in Addisonian pernicious anemia. To what is the value of yeast due? Castle and Strauss (9) identified the extrinsic factor with vitamin B_2 on the basis of the satisfactory results obtained upon administering to pernicious anemia

patients mixtures of gastric juice and autolyzed yeast. This contention, however, is being subjected to more or less dispute since Wills and others (10, 11, 12) have shown that the extrinsic factor cannot be vitamin B_2. Moreover, Greenspon (5) has introduced further doubt as to the identity of vitamin B_2 and the extrinsic factor. He has presented the concept that extrinsic factor is simply the activity that any protein has in forming adsorption compounds with pepsin and thus inhibiting any destructive influence it may have on other materials such as intrinsic factor. According to this view, the value of yeast would reside in its protein fraction. It is not conceivable that in the experiments herein described yeast owed its effectiveness to its ability to antagonize the effect of pepsin on the antipernicious anemia factor of gastric juice. There is less damage of the gastric mucosa in pernicious anemia of the tropics than in Addisonian pernicious anemia. According to Greenspon, therefore, yeast is more effective in the former condition because of a residuum of secreting gastric mucosa upon which it may act. It may act either by stimulating the cells that elaborate the gastric antianemia agent or provide material for its synthesis.

The present writer, as well as others (13, 14, 15) has shown that dogs suffering from a deficiency of the B complex are incapable of normal gastric secretory and motor activity. The appetite is poor, the food intake is reduced and what little is eaten is not nearly as well digested and absorbed as normally occurs. No matter how good the food mixture may be, under such circumstances several deficiencies may occur. Strauss (16) states that pregnancy definitely affects gastric function by reducing the amount of acid produced in the stomach. Rowland (17) adds that not only is the acid secretion diminished during pregnancy, but that there is also a temporary loss of intrinsic factor. The author has observed a similar reduction in hydrochloric acid in pregnant bitches. It seems to be clear therefore that in vitamin B (B_1) deficiency there is a marked reduction in the output of hydrochloric acid. This in itself will result in a severe impairment in the absorption of iron. It is not clear whether under these conditions other constituents of the gastric juice are diminished. Pregnancy is frequently complicated by a deficiency in vitamin B (18, 19). While such a deficiency is known to result in a reduction in gastric acidity, such a decrease occurring in pregnancy cannot be said always to be due to a shortage in the B vitamin. It may be that during pregnancy some other influence such as hormonal, may have an effect on gastric secretion similar to that of B avitaminosis. Nevertheless, such experiences as the preceding indicate that vitamin B plays an important rôle in the secretion of gastric juice and that this, in turn, is indispensable for the proper digestion and absorption of various materials concerned in the building of hemoglobin and erythrocytes.

Bethell and Sturgis (20) state that the specific antianemia property of

the vitamin B-complex is resident in the B_4 fraction and that this influences the synthesis of hemoglobin and the maturation of the red blood corpuscle. The results reported herein indicate that some factor in yeast provides not only for a marked increase in hemoglobin production but also for the development of the megaloblast into normoblast. It would seem from the above discussion that the value of yeast in promoting an increase in erythrocytes and hemoglobin under the conditions herein described lies in its content of vitamin B_1 and perhaps of vitamin B_4.

CONCLUSIONS

1. White rats raised on a diet devoid of the B complex and subjected to rarefied atmospheres are not able to compensate by an increased production of red cells and hemoglobin. The apparent increase in these factors is due to the anhydremia which develops during a severe deprivation of this complex.

2. Animals receiving the same diet supplemented by the addition of 2, 4, 6, 8, or 16 per cent brewer's yeast are enabled thereby to produce an increased amount of hemoglobin. As the amount of yeast increased, the diminution of red cells decreased. The largest inclusion of yeast provided for a definite increase in red cells. The increase in hemoglobin exceeded the increase in red cells.

3. The increase in the per cent of reticulocytes without a corresponding increase in the number of red cells represents a relative increase in the more immature forms and an increased destruction in the mature forms. As the amount of yeast supplement increased the per cent of reticulocytes bore an inverse relationship to the number of erythrocytes.

4. The red cells of animals exposed to decreased atmospheric pressure are capable of withstanding a saline solution of as low a concentration as 0.38 per cent. This represents a decreased fragility and may be considered as representing a mechanism compensating for the decreased production of red cells. This may be due to a cutting off of the splenic circulation.

5. Erythrogenesis of rats on the basal diet only seems to stop at the megaloblastic stage. Some factor in yeast appears to be necessary to carry the megaloblast to the normoblastic stage.

6. Reasons are assigned for believing that the chief benefits to be derived from yeast reside in the B_1 and B_4 fractions.

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THE EFFECT OF PROLONGED INANITION ON THE HEART WEIGHT/BODY WEIGHT (HW/BW) RATIO IN THE MAMMAL

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Jackson (1) in his book *Inanition and Malnutrition* makes a comprehensive review of the literature dealing with the effect of starvation on the heart weight. Investigation into Jackson's sources reveals that most of the workers quoted used only a very small number of animals in their work. His review serves only to emphasize the fact that there is no uniformity of opinion or evidence upon this subject.

Even today the most widely used textbooks of physiology still quote the work of Voit (2), done in 1866. He took two cats of equal weight, fed them equally for ten days, then killed one to serve as a control. The other cat was killed after thirteen days' starvation. He found that the loss in body weight was about 30 per cent, but that the heart had apparently lost only 2.6 per cent.

In 1895 Lazareff (3) reported work on a series of 80 guinea pigs. He used 40 animals to obtain normal data on the heart weight, and subjected the other 40 to starvation. The starved animals were divided into 4 equal groups, and these groups were so starved as to lose 10, 20, 30 and 36 per cent, respectively, of their body weight. The corresponding losses in heart weight were found to be 4.8, 9.1, 20.9 and 33.3 per cent, respectively.

Other workers, however, as shown by Jackson (1) reported other findings, ranging from no apparent loss in heart weight to a condition in which the percentage loss in heart weight greatly exceeded that of the body weight.

In consequence of the controversy upon the subject it was thought well to make an investigation of the matter.

METHOD. Guinea pigs were used in this work. They were selected especially for the purpose, and had not been used in other work. They were allowed water *ad lib*, but were fed a diet qualitatively adquate, but insufficient in quantity. After the animals had lost the desired amount of weight they were killed and the hearts removed. In each case the great

vessels were clipped flush with the heart, and the four chambers were opened and washed free of blood. The excess moisture was removed by blotting the heart with filter paper, and the weight of the heart determined. The heart weight/body weight ratio was found by dividing the heart weight, in grams, by the body weight, in kilograms. The normal heart weight/body weight ratio in the guinea pig was taken from previous work of Van Liere and Sleeth (4) in which the normal ratio was reported in a series of 77 male and 71 female animals.

RESULTS. Table 1 summarizes the results obtained.

It will be seen from the table that, in prolonged inanition, the heart loses slightly more weight than does the body. However, the difference in the percentage loss is so slight that it may well be in the range of experimental error, and no particular significance is attached to it.

TABLE 1

SEX	NUMBER OF ANIMALS	AVERAGE DURATION OF STARVATION	AVERAGE LOSS IN BODY WEIGHT	AVERAGE CALCULATED LOSS IN HEART WEIGHT	AVERAGE NORMAL HW/BW RATIO	AVERAGE HW/BW RATIO AFTER STARVATION	AVERAGE DECREASE IN RATIO
		days	*per cent*	*per cent*			*per cent*
Female	19	28	31	35	3.19	3.08	3.3
Male	14	24	29	34	3.17	2.99	5.6

DISCUSSION. We feel that the evidence presented above establishes conclusively that in starvation of sufficient duration to cause a loss of 25 per cent or more in body weight there is an approximately proportional loss in heart weight. These observations are in accord with those of Lazareff, previously mentioned, in his group of animals showing the greatest loss in body weight. These findings should be of interest to pathologists as well as to physiologists, since the determination of the heart weight is a routine part of every autopsy. Consequently, in such wasting diseases as tuberculosis or malignancy, where there is a marked loss in body weight, the post mortem diagnosis of small atrophic heart should be made only after this factor has been considered.

SUMMARY AND CONCLUSIONS

In a carefully controlled series of guinea pigs it was found that prolonged starvation had little effect on the normal heart weight/body weight ratio. It was shown that in cases in which the body had lost 25 per cent or more of its weight, the percentage loss in heart weight approximately paralleled the loss in body weight.

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THE INFLUENCE OF COPPER ON THE RATE OF DISINTEGRATION OF MAMMALIAN ERYTHROCYTES[1]

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The length of the life of red blood cells in the circulating blood is not definitely known. From the amount of bile pigments excreted, it has been calculated to be about three or four weeks. When the corpuscles reach a certain stage their cell membranes become weakened. This is followed by disintegration of the cell and liberation of the hemoglobin.

It is known that there is a decrease in resistance to disintegration of the corpuscles in certain diseases. Their resistance to hemolysis is thought by some to depend upon the age of the corpuscles, the membrane becoming progressively weaker with age. During recovery from hemorrhage the resistance of the corpuscles is greatly increased.

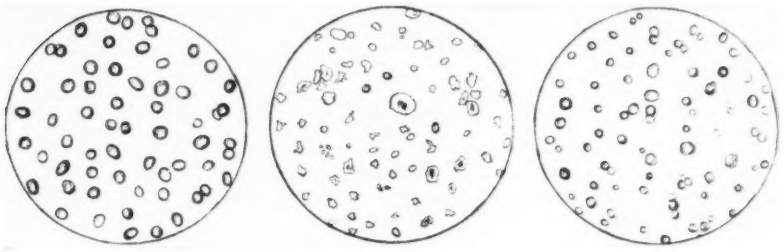
Animals given an iron-poor diet, such as milk, become anemic, and Hart, Steenbock et al. (1) have shown that the addition of iron alone to the diet is not sufficient to alleviate their condition, but that the addition of a trace of copper (about 0.2 mgm. per kgm. of body weight) resulted in correcting this artificially produced nutritional anemia. Recently Zondek and Karp (2) reported appreciably higher quantities of iron in many tissues with ageing, although the copper content under the same conditions did not show this variation. Broun (3) found that temporary anemia could be produced by vigorously exercising dogs that previously had been maintained under sedentary conditions. Low copper and iron intake diminished intracellular oxidation in the tissue of rabbits according to Locke et al. (4) and also lowered the copper level in the blood plasma. It was found that the rate of disintegration of erythrocytes was markedly decreased when a small amount of copper sulphate was added to the cells in vitro (5).

The present investigation was undertaken in order to secure further information regarding the relative importance of copper on the rate of disintegration of erythrocytes of animals during recovery from nutritional anemia.

EXPERIMENTAL. Thirty albino rats, 6 to 8 weeks old, were used in these

[1] A preliminary report of this paper was given at the meeting of the American Physiological Society, March, 1934.

experiments. Twenty of the animals had been made anemic by placing them on a milk diet. Hemoglobin determinations varied from 2.5 to 7.0 grams per 100 cc. of blood with an approximate average of 5.0 grams for the group. The animals were divided into two similar groups. To one group was given daily, unskimmed cow's milk and ferric citrate equivalent to 0.5 mgm. iron. The remaining ten animals were given daily, in addition to the milk and ferric citrate, 0.025 mgm. copper as copper sulphate. Ten animals used as controls were raised on a stock diet. After a ten day feeding period, blood samples were taken. The animals were rendered unconscious by a blow on the head, the thoracic cavity immediately opened and about 2 cc. of blood were obtained aseptically by cardiac puncture and placed in sterile tubes. The tubes were gently shaken to prevent formation of a firm clot, then placed in a moist chamber at room temperature (about 20°C.) and the erythrocytes were permitted to disintegrate in their



A B C

Fig. 1, A, B, C. Camera lucida drawings of erythrocytes of normal, anemic and copper treated rats.

own serum. Daily microscopic observations of the red blood cells were made.

Four days after blood samples were taken, camera lucida drawings were made of typical erythrocyte suspensions. It will be seen from figure 1, A, that some of the erythrocytes from the control animals are showing irregular outlines, indicating beginning disintegration. Figure 1, B, shows that the cells of the anemic animals given milk-iron diet are almost completely disintegrated in the same period of time. Cells in figure 1, C, are from the milk-iron-copper fed animals and show disintegration had not begun and continued observations revealed that it did not begin until several days later. It will be seen that these cells are not uniform in size, but their membranes are remaining intact. Anisocytosis, or variation in size, is frequently a manifestation of an accelerated formation of blood.

To determine if this great variation in rate of disintegration of the cells

was due to difference in sera or to the erythrocytes themselves, the following experiment was performed. Erythrocytes of anemic animals placed in the serum of normal or copper-fed animals showed the rate of disintegration identical with the rate in their own serum. Similarly, the cells of copper-fed animals were introduced into the sera of anemic and normal animals. These erythrocytes showed the same prolonged resistance as they did in their own serum.

Although copper has not been found a constituent of the hemoglobin molecule, Elvehjem, Steenbock and Hart (6) found that in a given quantity of whole blood, the corpuscles contained more copper than the serum.

SUMMARY

1. The rate of disintegration of erythrocytes of nutritionally anemic rats was found to be greater than that of normal animals.

2. Erythrocytes of animals given an adequate copper diet showed increased resistance to disintegration, and these experiments would seem to indicate that a beneficial effect of copper could be attributed to changes in the corpuscles.

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A NEW METHOD OF PARTITIONAL CALORIMETRY

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We present here a new method of calorimetry which seems peculiarly adapted for the study of body heat elimination and temperature regulation. From a theoretical standpoint its chief characteristics, as contrasted with the use of static or "heat trap" calorimeters, are two. In the first place, it makes possible a record of thermal interchanges as they occur *within a brief interval of time* instead of summing up results extending over a period of several hours, in which the process of adaptation is masked by cumulative averages. In the second place, its data are given in terms of rates of thermal interchange partitioned by primary measurement in accordance with the physical avenues through which the interchanges occur.

For the procedure in question, we employ the term *partitional calorimetry* to distinguish it clearly from the conventional form of static calorimetry in which the primary measurement consists of a quantity of heat "captured" by the apparatus.

Direct calorimetry of the ordinary type is not adapted to a study of the biophysics of heat elimination when experimental interest demands an accurate partition of the energy metabolism. This arises from the fact that the direct calorimeter affords no opportunity for the separate determination of the values of radiation and convection loss. In addition, the temperature range over which such apparatus can operate is limited, in practice if not in theory, by the labor involved in determining the constants of a calorimeter for more than one condition. Furthermore, long-time observations in an ordinary calorimeter can only be conducted under conditions within a fairly narrow range of physiological adaptability; while for the shorter periods required by our procedure the subject can endure more extreme ranges of heat or cold.

A generalized biophysical treatment of the problem would not be greatly furthered even by a complete and accurate partition under a single standard environmental condition. Such a generalized treatment of the problem can be furthered only by empirical partitions over a wide range of conditions, incorporated in a methodological framework which permits the derivation of the equational forms and constants necessary for a

general solution. In the briefest possible terms, the end which we have sought is the determination of the several functional relations which will make possible the computation of the "probable calorie demand" of a given physical environment upon an organism of any given physical and thermogenic proportions, and the partition of that demand between the various avenues of heat loss.

The need for such a treatment can be convincingly demonstrated either by reference to the theoretically unsatisfactory character of the equations now used in the estimation of normal metabolism, or by illustrations from the literature concerned with physiological responses to heat. In the latter case the possibilities of describing the thermal stimulation of the environment are practically exhausted when the dry and wet bulb temperature and air movement have been recorded. Aside from the fact that such a record is inaccurate to the extent to which radiation exchange is neglected, it is also dimensionally primitive. Many combinations of wet and dry bulb temperatures exist which are physiologically, as well as psychologically, isothermal (Houghton, et al., 1930). The lack of an objective measure of total calorie demand hampers the comparison and evaluation of an enormous body of experimental data. It is precisely as if we were engaged in an experiment in which the critical stimulus was the volume of a rectangular solid, but being ignorant of the concept of volume, our stimulus was constantly recorded in terms of its three varying linear dimensions.

Partition of the heat loss from the human body into its separate physical components has been made by a number of investigators. Rubner's partition (1896) is the most frequently quoted. The components were not experimentally determined, and the partition applied to only one atmospheric environment. It was not intended to be more than a rough approximation. Aldrich (1928) improved the situation by determination of the radiation loss but estimated evaporative loss and obtained the convection component by difference. The excellent work of Houghton, Teague, Miller and Yant (1930) determined a partial partition over a range of temperatures but did not develop a method of separating radiation and convection. Bohnenkamp (1931) studied radiation in detail but estimated both evaporation and convection. As evaluated from the standpoint of the desirability of a method of complete partition which could operate over a large range of conditions, all of these studies failed to achieve empirical completeness in that no technical means of fractionating the radiation-convection component was devised.

The value of the method of "partitional calorimetry" described in the present paper lies not merely in the determination under given conditions of the important fractions in heat elimination, but also in the demonstration of the varying proportions of the total which single factors assume as

conditions are varied over a wide range. When such manipulation and measurement are possible it becomes feasible through proper mathematical treatment to determine the effective radiation surface of the human body, the constants of the equations which govern conduction and convection loss for any difference between ambient air and radiating surfaces and body surface, and the changes in these relations with variations in posture. Ultimately it makes possible the determination (within certain limits) of metabolism, without resort either to respiratory measures or direct calorimetry.

Data required in differential partition of heat loss. The values of the principal fractions of heat loss from the human body at rest[1] are determined by the evaporative processes (E), the infra-red radiation exchange between body and environment (R), and the conductive-convective exchange (C) between the ambient air and the body surface. The important factors governing these several exchanges classified according to their location (i.e., as measures of characteristics of the environment and of the body respectively) are indicated below. In this analysis we consider the body, whether clothed or unclothed, as a unit. Therefore, the "areas" considered may be areas of skin or of the mucosa of the respiratory passages or of the clothing. The areas contributing to evaporation, radiation and convection obviously need not be the same.

I. Evaporation:
  a. Environment
    1. Dry bulb temperature of ambient air.
    2. Degree of movement of ambient air.
    3. Relative humidity of ambient air.
  b. Organism
    1. Mean moisture available[2] per unit area on effective evaporating surfaces.
    2. Area of such evaporating surfaces.

II. Radiation exchange:
  a. Environment
    1. Radiant topography of environment.[3]
  b. Organism
    1. Mean temperature of external surfaces of the body system.
    2. Effective radiation area.
    3. Radiation absorption characteristics of such surfaces.

[1] Ignoring for the moment the small fractions due to warming of food or drink, heat equivalent for the excreta, and warming of inspired air.

[2] The term "moisture available" is purposely chosen to emphasize the physical viewpoint, and to avoid *at this point* a discussion of the exceedingly complex physiological variables which determine, in part, this factor.

[3] The effective radiant temperature of surrounding surfaces may be taken as a measure of this factor.

III. Convection:
  a. Environment
    1. Dry bulb temperature of ambient air.
    2. Degree of air movement.
  b. Organism
    1. Mean temperature of external surfaces of the body system.
    2. Effective convection area.

The general factors given above which condition the energy interchange in this physical system (of body and environment) are obviously indicated by the established principles of physical science. The useful application of data representing these variables is, however, entirely dependent upon the determination of the heat loss for any one of these three avenues, given the measurements associated with it in the preceding analysis. In the practical solution of this problem and the primary calibration of the method it is necessary to consider not only the heat loss but the heat production of the organism. The measurement of metabolic rate is, of course, required, and in addition changes in the total heat content of the organism must be assessed.

Storage as represented by a shift in the theoretical mean temperature of the entire body is not in a category uniform with heat exchange by evaporation, radiation and convection, since it does not involve a direct exchange of heat with the environment, but rather a change in the energy state of the body itself. In a dynamical treatment of heat balance between the metabolic rate and the environment it is, however, necessary to include storage which represents the heat absorbed or lost by the body. It is obvious that if our sole interest is in the relative partition of heat under given environmental conditions, the simplest procedure is to work with subjects in thermal equilibrium, at which point the value of the storage rate is zero. Our experience, however, has constantly impressed us with the probability that the most significant phases of physiological responses to thermal stimuli are missed if study is limited to equilibrium cross sections. To this end our attention has been largely directed toward perfecting the method of partitional calorimetry for use during either transitional or equilibrium stages.

In addition to the three groups above, we may add a classification of the important storage factors, as follows:

IV. Factors influencing heat storage.
  a. Environmental
    1. Total calorie demand of the environment (as influenced by all the factors listed under I, II, III).
  b. Organism
    1. Change in rate of metabolism.

The heat stored in the body must obviously depend on the net difference

between these two factors. It is generally measured by changes in the rectal body temperature but, as might be expected, and as our studies will show, this measure may give a very inadequate picture of actual heat storage by the body as a whole. The classical methods of calorimetry can only be applied when adaptation has brought about a correspondence between rectal temperature and the temperature of all organs; but by partitional calorimetry this factor may be evaluated by difference over relatively short periods of time and while the adaptation is actually in process.

It is clear that our elementary data of observation must be related to mathematical forms which are competent to express the thermal interchange through the several avenues which account for the total heat loss. The general type of expression for each avenue, as based on the usual experimental measurements, may be briefly indicated as follows:

I. Evaporation:

H_2O loss in grams per unit-time $\times$ Lat Heat of evaporation.

II. Radiation:

A constant $\times$ the difference between the fourth powers of the absolute temperature of the skin and of the absolute temperatures of surrounding surfaces $\times$ effective radiation surface area.

III. Convection:

A function of skin temperature, air temperature and air velocity.

Our chief objectives are the determination of the effective radiant surface area in II, and of the functional type and constants involved in III.

The function describing the radiation exchange per unit area (Stefan's Law) is theoretically competent and entirely general. In computing the total radiation exchange, however, it is extremely difficult to determine, except for regular and symmetrical situations, the "effective radiation surface" (in our case, of the human body). This is true because the effective radiant surface is covariant with every change in the relative geometrical orientation of the surface of an object with regard to its enclosure. Hence, an important experimental aim is the determination of "effective radiation surface area."

In the case of convection, we recognize that this exchange is implicitly a function of the three variables noted. We have, however, no explicit statement of this function comparable to Stefan's equation for radiation. It is sufficient to note that equations of use in describing convection loss under ordinary experimental conditions are empirical in nature, and are in the sense of theory, incompetent for generalized use. In the case of radiation exchange we are forced to determine a variable difficult in measurement, the effective radiation surface area (S_r), for use in an explicit function. The convection problem, on the other hand, demands the determination of constants for an empirical equation, whose chief variables

may be measured with relative ease. While the evaporative loss is easily determined by measurements of weight loss, the solution of the problem mentioned above demands a methodology by which radiation and convection may be separately measured over an area of variation in their respective magnitudes sufficiently large to allow, from the empirical results, determination of the proper factors needed for a general solution. Hitherto this partition has been impossible because under ordinary experimental circumstances the radiant temperature of the environment and the

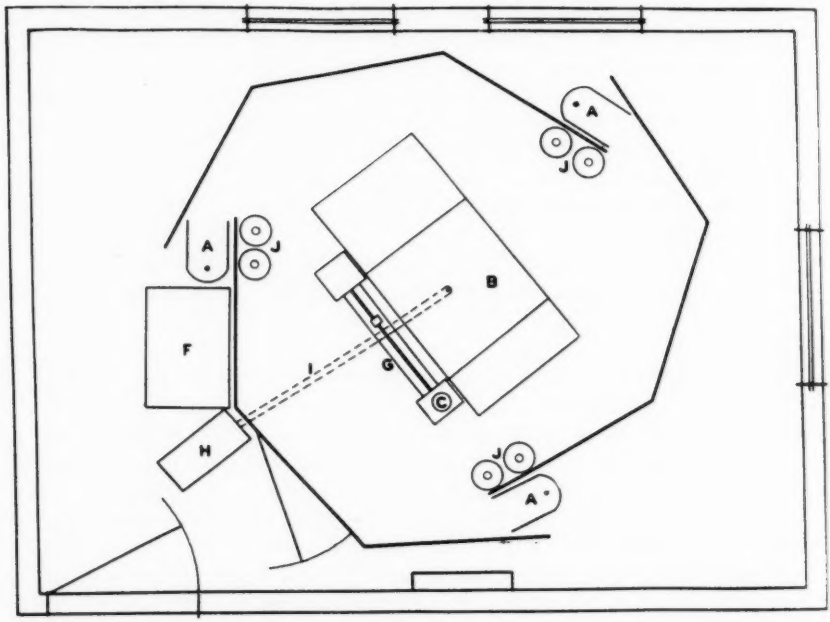

Fig. 1a

Fig. 1a and 1b. Equipment for study of thermal interchanges by the method of partitional calorimetry. *A*—2500 watt heaters; *B*—chair; *C*—aspirating psychrometer; *D*—Moll thermopile; *E*—copper hemisphere (radiometer); *F*—chart table; *G*—platform scales (balancing beam in *a*, platform in *b*); *H*—Benedict-Roth metabolism apparatus; *I*—hose connection to metabolism apparatus; *J*—six-inch fans directed to floor of booth; *K*—reference temperature bath for rectal thermocouple.

ambient air temperature assume closely related values, so that the fractions due respectively to convection and radiation are experimentally indistinguishable.

The difficulties in the way of a complete partition are overcome in the method of partitional calorimetry by placing the subject in a nine-sided copper enclosure (see fig. 1). Properly located radiant heaters, A, in-

troduce into the enclosure infra-red radiation which is reflected and diffused over the entire area as a result of the high reflectivity coefficient of the copper surfaces. Scarification of the reflecting surfaces prevents image reflection and results in a mean radiation intensity within the enclosure which is remarkably uniform in all directions. The enclosure is freely open to circulation of air from the exterior, above and below the radiant heaters. Thus, the temperature and humidity of the air within the enclosure is only slightly above that of the air-conditioned chamber in which

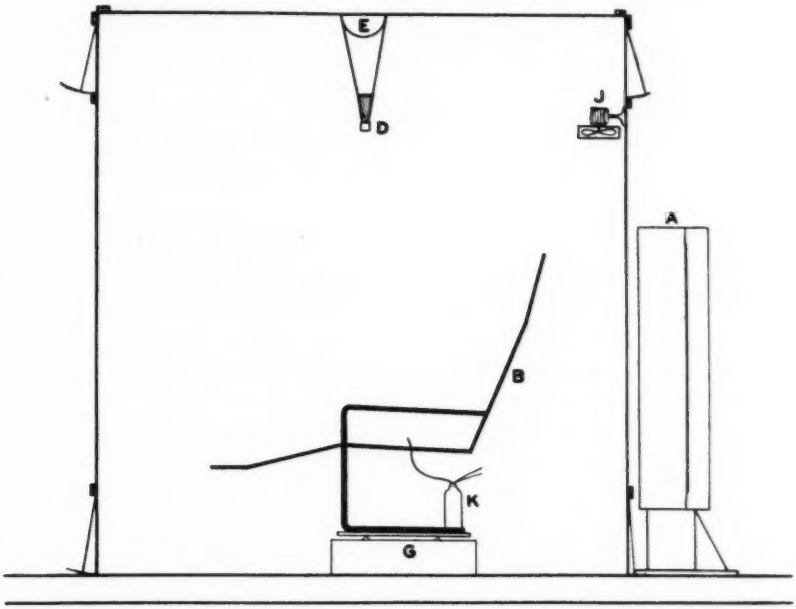

Fig. 1b

it is located, while the environmental influence, so far as radiation is concerned, is governed by the optical temperature of the copper surfaces which completely surround the subject.

Under these circumstances it is easy to vary the mean radiant temperature of the surfaces surrounding the subject independently of the ambient air temperature. With an ambient air temperature of 15°C., it is possible to produce radiant temperatures within the enclosure with any desired value between 15° and 55°C. It is perhaps well to point out that while this radiant temperature is physically real in its effect upon the radiation exchange, the actual contact temperature of the copper walls is at, or very near, the ambient air temperature. The hand placed near the metal

surface perceives a sensation of heat characteristic of that emanating from a black body at, let us say, 50°C. If the ambient air temperature in the enclosure is 15°C., actual contact with the surface produces a temperature sensation characteristic of a metal surface at 15°C. In a sense, then, the radiant temperature of the enclosure is an "optical" temperature, independent of the contact temperature. This point is important for the consistent functioning of the apparatus at various radiant temperatures far removed from the ambient air temperatures, as it prevents the production of strong convection currents over the surface of the metal. If this were not so, the convection loss in any condition would be greatly influenced by the auxiliary air turbulence characteristic of the temperature difference between metal surfaces and ambient air.

The importance of this technique of rendering the environmental radiant temperature independent of the environmental ambient air temperature cannot be overemphasized. Upon it, the fruitfulness of the method is entirely dependent.

From a theoretical point of view it is obvious that the provision of technical means of independently adjusting the values of the mean radiant and ambient air temperatures immediately provides a method of separating the convection and radiation losses from the human (or animal) body. The radiation exchange is controlled by the relative values of the skin temperature and the mean radiant temperature of the enclosure; in like manner the convection loss is a function of the relative values of the skin and ambient air temperatures. Since the algebraic sum of the metabolism, storage and evaporation must be balanced by radiation and convection, it is obvious that an elimination of any loss or gain by one of these two avenues (radiation or convection) will give directly the value of the loss conditioned by the other variable.

The range of such experiments is necessarily limited by the endurance and thermal adaptability of the subject. For example: with a radiant temperature of 27°C., if we desire to maintain a skin temperature of 27°C., so as to balance out radiation loss, the subject would have to be exposed to an environmental air temperature of approximately 0°C. This is a somewhat rigorous condition for the nude human subject. However, such a limitation applies chiefly to experiments in which radiation must be adjusted for theoretical purposes to the value of the mean skin temperature. An air temperature of 0°C. can readily be made bearable by raising the mean radiant temperature to 45°C. Such conditions produce interesting physiological responses.

It will be seen in later papers from this Laboratory that a practical, satisfactory compromise may be reached by varying the convection and radiation terms in such a manner that not only are the unknown factors (effective radiant surface area and the convection function) determined, but the conditions are kept within an endurable range.

The detailed technique employed in our studies is described in succeeding paragraphs.

I. *Calorimeter.* The copper enclosure consists of three sections of three panels each, each panel being 38 inches wide by 8 feet high. The sections are set up on a copper floor and staggered to give a floor arrangement such as is shown in figure 1. A copper ceiling caps the three sections, forming a small room 8 feet in height with a capacity of 500 cubic feet. The stagger allows the insertion of three electric radiant heaters, A, at the points indicated in figure 1. The resulting radiation is diffuse and the intensity relatively uniform in all directions.

II. *Radiation source.* Each of the three electric heaters (*A* in fig. 1) consists essentially of a 4-foot Nichrome coil of 2500 watts capacity located at the focus of a section of a copper parabolic cylinder acting as a reflector. Copper side wings, 10 inches wide, attached to the sides of the section act as blinkers and further direct the radiation into the booth enclosure. Each heater is controlled by a rheostat and is so wired as to allow a convenient measurement of the wattage input.

III. *Control of atmospheric conditions.* The copper enclosure is located in an air-conditioned chamber, surrounded by air-conditioned shell-spaces. (Test room 1, Winslow, Greenburg, Herrington, and Ullman, 1934.) Independent conditioning equipment is available for both chamber and shell-space. In the experiments here described only the shell-space conditioning system was used, since it is far more delicate in its control than the system serving the chamber itself. Windows between chamber and shell-space were kept open so that the chamber air took on the temperature of the shell-space air and the air in the copper booth took on essentially the temperature of the chamber air, as described above. Our conditioning equipment allows the maintenance within the shell-spaces of dry bulb temperatures between $-10°$ and 55°C., with an accuracy of $\pm.3$°C. This control results in a range of ambient air temperatures within the copper enclosure (with radiant heaters in operation) of 5° to 60°C., without local forced ventilation within the booth itself. Relative humidity may be regulated over a range from 15 to 95 per cent with a high degree of accuracy. Measurements of air movement within the copper enclosure obtained with a hot-wire anemometer, when no local forced air movement was employed, indicate values from 15 to 25 linear feet per minute, due to convection currents, a condition which is comparable with the "still air" of an indoor space not provided with forced ventilation. By regulating the conditioning equipment so as to obtain the lower limit of dry bulb temperature in the enclosure (5°C.) and setting the radiation equipment for the total maximum output (7500 watts), a difference of 40° between the temperature of the ambient air and the mean radiant wall temperature (45°C.) of the enclosure may be produced.

IV. *Subjects and circumstances under which they were observed.* a. *Sub-*

jects. Two subjects were used for the experiments reported in the present papers. One was of the slender, leptosomic type, the other of the stout, pyknic type. Their physical characteristics will be discussed in a later paper. They were first subjected to a series of preliminary experiments to accustom them to the routine, and actual experimentation was conducted for a period of about eight months.

b. *Conditions with regard to clothing.* All the data here reported were obtained with the subjects unclothed except for an athletic supporter. The behavior of clothed subjects will be studied in later investigations.

c. *Posture.* All our data so far have been obtained with the subject in a seated posture with the feet on a support so as to be nearly horizontal. The chair used (fig. 1, *B*) was of steel-frame construction with a seat of canvas and an open back, except for a narrow strp of canvas across the waist and another canvas band to support the head. The chair rested on a delicate silk scale to record evaporation loss.

V. *Recording experimental conditions.* a. *Ambient air temperature.* The ambient air temperature in the booth was continuously measured on the time chart of a Leeds and Northrup recording potentiometer. The sensitive elements are 6 iron-constantan thermocouples wired in parallel and arranged in three tiers of two each. The location of the three tiers corresponds to shoulder, mid-trunk, and knee positions of the seated subject. The sensitive elements themselves were made of very fine wire, no. 30, spot welded. The resulting couples have very small heat capacities and surface area, and record air temperature within a half a degree without an appreciable error due to radiation.

b. *Relative humidity.* The relative humidity of the air in the copper booth is determined once an hour by an aspirating psychrometer, *C*, which yields results accurate within two per cent.

c. *Air movement.* With the low velocity employed, air movement is of a turbulent character and varies widely from point to point. At a given point we can determine the existing velocity very accurately by the use of a special hot-wire anemometer described in a previous paper (Winslow, Gagge, Greenburg, Moriyama and Rodee, 1935). To estimate the mean velocity affecting the subject we made observations over 15 points on the body at a distance from the body surface of 6 inches. The location of these points is shown in figure 2. A weighted mean value was determined from these observations as described in a later paper.

d. *Mean radiant temperature of the enclosure.* The mean radiant temperature of the walls of the copper enclosure is measured by a Moll 80-element thermopile (fig. 1, *D*) whose optical field is the surface of a copper hemisphere of 6 inches diameter (fig. 1, *E*) which is suspended from the ceiling of the enclosure. The copper hemisphere acts as a reflecting surface which samples the radiation from the various areas of the enclosure and

renders the pile sensitive to their multi-directional radiation effects. The e.m.f. delivered by the thermopile under these conditions is directly convertible into the mean radiant temperature of the enclosure by means of a calibrated relation previously determined for our physical situation. The exact method of calibrating the instrument is fully described in a previous publication (Winslow, Gagge, Greenburg, Moriyama and Rodee, 1935).

VI. *Measurements of physiological variables.* a. *Mean skin temperature.* This value is determined by measurements of the mean temperature of 15 circular skin fields one and one-half inches in diameter. The term "skin field" is used, since our method employed a thermopile which, of course, involved no contact with the skin, and gave a mean value for the area surveyed. In actual measurement, the pile was held directly over the skin field being examined. The distance of the pile elements from the skin surface (and hence the area surveyed) was held constant by a simple feature in the construction of the instrument which can best be seen in the original description by Hardy (1934). In order to reduce the temperature topography of the body to a mean value, properly weighted for regional differences in both surface area and local temperature, we computed mean temperatures for four anatomical segments; the head, the upper extremities, lower extremities and trunk. Each segmental mean temperature was then weighted by a factor numerically equivalent to the percentage of the total surface area represented by the separate segments. The percentages were taken from DuBois (1916). The resulting mean skin temperature obtained is the datum which enters into both radiation and convection computations. The location of the 15 skin fields was the same as that indicated for measurements of air movement in figure 2. In making these observations the thermopile was manipulated by the subject himself so as to avoid the effect of an observer upon the thermal conditions within the booth while the records were read on a galvanometer outside, *F*.

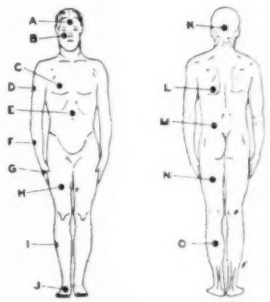

Fig. 2. Fields used for air movement and skin temperature observations. Head, *A*, *B*, *K;* upper extremities, *D*, *F*, *G;* trunk, *C*, *E*, *L*, *M;* lower extremities, *H*, *I*, *J*, *N*, *O*.

b. *Evaporative weight loss.* A determination of the weight loss of a subject was made at intervals of 20 minutes by reading the scale upon which the chair of the subject rested. The sensitivity of the scale, *G*, used was 2 grams. Frequent repetitions of the measurement on a subject in equilibrium established a linear rate of loss, the accuracy of which

is not substantially improved by more sensitive apparatus. This fact having been established, actual choice of the platform scale instead of the highly sensitive Sauter balance (sensitivity 50 mgm.), was determined entirely by the fact that the former allows postural conditions to be varied with ease, and is relatively insensitive to building vibration or air waves set up by air-conditioning equipment. A correction factor is applied to the loss in grams to compensate for the weight inequalities of the O_2 and CO_2 components of the respiratory exchange. This correction is based on an R. Q. of 0.83 and the observed O_2 consumption.

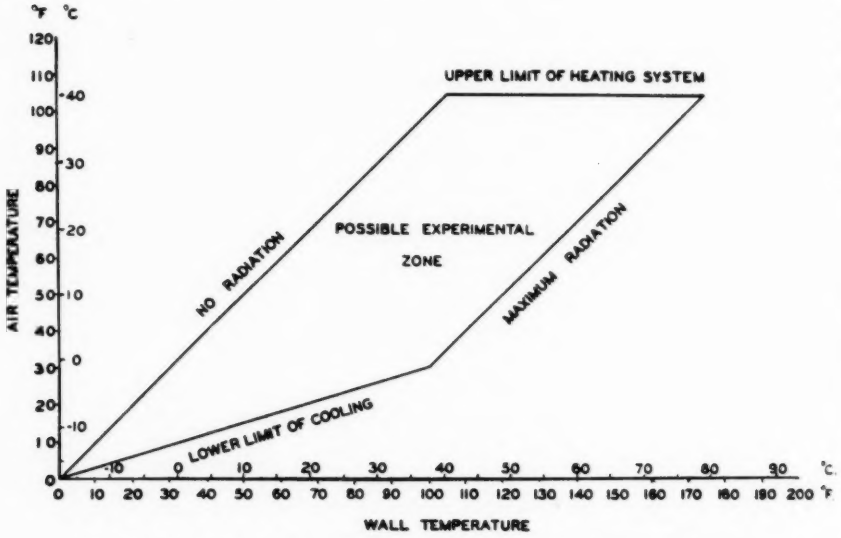

Fig. 3. Range of air and wall temperatures obtainable in copper booth

c. *Determination of metabolic rate.* The measurement of heat production was accomplished indirectly by determination of the O_2 consumption with a Benedict-Roth metabolism apparatus, *H*. Connection was made from the subject with this apparatus by the hose, *I*. No attempt was made to attain basal conditions, although strictly basal measurements were separately made on each subject as a part of the routine. Emphasis was put rather upon standard conditions. Experiments were conducted after 9 a.m. and after 1:30 p.m. Breakfast and luncheon of moderate proportions were allowed, and excessive walking and exercise prior to the tests prohibited.

d. *Measurements related to heat storage.* Rectal temperatures could be taken at any time by means of a thermocouple imbedded in a gold-plated

applicator which was inserted for a distance of six inches beyond the anal sphincter and kept in place throughout the experiment. Oral temperatures were taken at regular intervals. Such measurements are invaluable as indicators of equilibrium conditions and, within limits, they are of use in the computation of heat storage when balances are computed during transitional states. All temperatures were automatically recorded outside the booth.

VII. *Experimental routine.* In sections IV and V we have described briefly the instrumental background of our technique for partitional calorimetry. One hour, or longer if necessary, before the experiments begin, the conditions are set up so that complete equilibrium exists before the subject enters the booth. The nude subject is then seated on a light metal chair on the platform of a scale located at the center of the booth. The routine of observation, arranged progressively in time, is illustrated below. The fans, *J*, provided for producing forced air movement, were not used in the experiments reported here.

PART A

p. m.	*Official time in minutes*	
1:30		Subject arrives at laboratory, disrobes.
1:45		Subject enters calorimeter; is seated and rectal thermometer inserted.
1:50	0	First weight determination, rectal and oral temperatures and relative humidity.
2:10	20	Second weight; rectal and oral temperatures.
2:30	40	Third weight; rectal and oral temperatures.
2:50	60	Fourth weight; rectal and oral temperatures.
2:50–3:05	60–75	Metabolism; official wall and air temperature readings simultaneous with skin temperature series.

PART B

3:05	75	Fifth weight; rectal and oral temperatures, relative humidity.
3:25	95	Sixth weight; rectal and oral temperatures.
3:45	115	Seventh weight; rectal and oral temperatures.
4:05	135	Eighth weight; rectal and oral temperatures.
4:05–4:20	135–150	Metabolism; official wall and air temperature readings simultaneous with skin temperature series; final relative humidity.
		Subject released.

The air temperature and the wall temperature are continuously recorded throughout the experiment but the readings used for our partitions are only those that are taken simultaneously with the skin temperature. The relative humidity is recorded three times during the experiment. A time record is made continuously throughout the experiment of the weight losses and rectal and oral temperatures.

Limitations of apparatus. In this connection a brief consideration of the actual thermal limits of the apparatus is in order. In figure 3 we present a chart in which the possible combinations of radiation temperatures and ambient air temperatures are designated. The heavy diagonal lines enclose the actual areas within which we can produce the radiant and ambient air temperatures which appear respectively as the coördinates of any point. It is clear that the method of partitional calorimetry in its exploratory form does not enjoy, within the limits of a single experimental session, the classic accuracy of direct calorimetry. Certain refinements in the apparatus, more frequent determinations of metabolism and skin temperature, the introduction of a long period of thermal preadaptation, and the extension of the experimental period in time, would serve to reduce errors in energy balance.

The higher random error present in such studies as ours, as compared with those present in direct calorimetry, is balanced by the partitive measurement of heat loss and by the possibility of obtaining data during transitional physiological states. This is particularly true since our objective has not been the measurement of metabolism, but rather the determination of the mathematical forms which must relate empirically observed heat production to heat loss in terms of physical variables in the thermal environment and animal body. Its eventual possibilities as a new form of direct calorimetry should not, however, be lost sight of because of our deliberate sacrifice of immediate accuracy in the interest of a reasonably rapid survey of a wide variety of conditions pertinent to our objectives.

SUMMARY

1. A brief survey has been made of typical physical partitions of heat loss from the human body presented by earlier observers.

2. The point has been made that these partitions have failed to yield any theoretically competent insight into the physical relations which govern heat loss, largely because they do not represent in any case experimental attempts to obtain simultaneous measurements of all factors. Only one treatment (Houghton et al., 1930) has utilized the obvious analytic advantages of a series of experiments made over a comparatively wide range of thermal conditions, as contrasted with intensive studies at one, or a few standard conditions.

Furthermore, in no case has a technique been developed for the experimental variation of the radiation factor independently of the convection factor.

3. A method of partitional calorimetry is described which is distinctive in the sense that it permits for the first time a theoretically competent treatment of the energy relations between body and environment as they

are involved in the transfer of thermal effects over separate energy avenues. This end is achieved through a procedure which permits an independent variation of radiation and convection over a wide range, and yields data which possess the analytic advantages associated with simultaneous variation and partitive determination of the values of each variable in the energy system.

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THE LINEARITY CRITERION AS APPLIED TO PARTITIONAL CALORIMETRY

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General theory of the partition of energy metabolism. In the course of our studies of thermal interchange between the human body and its environment, it was important to determine the range within which our physiological observations could be relied upon as representing true relationships to the physical conditions surrounding the body—uncomplicated by serious experimental errors or physiological reactions other than those primarily postulated. The following method of analysis has been found satisfactory for this purpose.

When no work is being done on or by the body, the methods by which the metabolic energy may be dissipated are fourfold; namely, Convection, C, Radiation, R, Evaporation, E, and Storage, S. The algebraic sum of these terms must equal in magnitude the energy Metabolism, M. The first law of Thermodynamics may be expressed by the equation

$$M \pm S - E \pm R \pm C = 0, \tag{1}$$

where the convention in signs is so chosen that the sign of M is always positive. Storage, S, with a positive sign, indicates a lowering of body temperature; with a negative sign, a raising of body temperature. Evaporation, E, has always a negative sign. Radiation, R, is positive when the surrounding wall surfaces are of higher temperature than the body surface; negative when these surfaces are of lower temperature than the body surface. Convection, C, is positive when the surrounding air temperature is higher than body temperature and negative when this temperature is lower than that of the body surface.

Equation (1) may be written in the detailed form

$$M - \sigma_B T'_B - Lw + A_R k_R (T_W^4 - T_S^4) - C(T_S - T_A, V) = 0. \tag{2}$$

Let us consider each of the terms of this equation separately.

The first term is the Metabolism, M and is measured by the O_2 consumption and CO_2 loss. It represents the total power created by the body and it is this energy rate for which the partition is actually made.

The body creates no other form of energy that cannot be accounted for by the normal processes of O_2 consumption.

The Storage, S, is directly proportional to the time rate of change of the mean body temperature T_B, which is written T'_B. The constant of proportionality, σ_B, is dependent on the total body weight and its specific heat. Further, according to the convention of signs stated above, S is negative when T'_B is positive and vice versa.

The Evaporation loss, E, is directly proportional to the net rate of weight loss, w of the body, after correction for O_2 gain and CO_2 loss. The constant of proportionality, L, is the latent heat of evaporation of H_2O at normal skin temperatures.

The Radiation term, R, is given by the simple Stefan-Boltzmann equation. A_R is the effective radiation area of the body. k_R is the universal radiation constant. T_w is the mean black body radiant temperature of the environment with reference to the position of the subject in absolute degrees, and T_S is the mean skin temperature in absolute degrees also. The skin is assumed to have black body characteristics and the associated angle factor is unity on account of the particular choice of wall temperature, T_W.

The Convection term, C, is written in equation (2) as a function of the difference between the mean skin temperature, T_S, and the ambient air temperature, T_A, and of the average air movement, V, about the body. The convection, C, is always negative when the air temperature is below the skin temperature.

In general, of the terms in equation (2) we see the basic physical measurements are T_W, T_A, and V. The basic physiological measurements are M, T'_B, T_S, and w. The measurement which requires separate experimental determination is the area, A_R. Further, the functional form of C is not definitely known. Thus, the value of A_R and the form of the function, C, are the unknowns that prevent a complete partition of the energy metabolism from the basic measurements alone.

In the radiation term let us write

$$R' = k_R(T_W^4 - T_S^4). \tag{3}$$

The value of R' may be calculated from its basic measurements. Introducing (3) in (2) we have the simplified equation

$$(M \pm S - E) + A_R R' - C(T_S - T_A, V) = 0. \tag{4}$$

In the application of actual physical measurements to (4) one finds that the validity of the equation depends on two assumptions: (a), the storage is measured accurately by the rate of change of the body temperature as actually recorded, e.g., orally or rectally; (b), the weight loss of the body (corrected for O_2 and CO_2 changes) gives completely the energy

equivalent of the evaporation loss (i.e., 100 per cent evaporation efficiency). Only if these assumptions are valid may one be justified in any conclusions that may follow concerning the value of the area, A_R, and the nature of the convection function, $C(T_S - T_A, V)$.

The linearity criterion and its demonstration. If the two assumptions mentioned above are not true the resulting values of metabolism, storage, and evaporation as determined from their basic measurements will be incorrect and will differ from their true values by the amounts ϵ_S, and ϵ_E respectively. These error terms, ϵ, if present, may be constants, or may vary with the experimental conditions. Equation (4) now becomes

$$M + (S + \epsilon_S) - (E + \epsilon_E) + A_R R' - C(T_S - T_A, V) = 0, \quad (5)$$

where the convention in signs is so chosen that each measured value must be increased by a quantity, ϵ, in order to represent the true value. The corrections, ϵ, represent *inherent errors of measurement* rather than purely random errors which affect only the deviation of individual points from a normal curve.

If the error terms were all zero then the algebraic sum of $M \pm S - E$ would be the total energy that must be balanced by radiation and convection. For convenience we shall describe the sum of the terms, $M \pm S - E$, by H, which, to draw a physical analogy, is the net heat output by the body that should be partitioned into radiation and convection. Then, rewriting (5), we have

$$H + (\epsilon_S - \epsilon_E) + A_R R' - C(T_S - T_A, V) = 0. \quad (6)$$

Let us choose a set of conditions such that C is constant (i.e., hold $T_S - T_A$ and V constant), and vary R' (i.e., T_W) over a large range of both positive and negative values. If we plot H on the ordinate versus R' on the abscissa for each of the experiments under these conditions we shall obtain a straight line if, *and only if*, the error terms are zero, or constant.

Experiments with radiation parameter. Experimental equipment: A copper booth was used as a means of obtaining widely differing wall and air temperatures. The methods of making the physiological measurements (metabolism, storage, evaporation and skin temperature) and the physical measurements (wall temperature, air temperature and skin temperature) have all been described in a preceding publication (Winslow, Herrington, and Gagge, 1936).

Experimental conditions: Two series of experiments were carried out; the first series was so chosen that $T_S - T_A$ was about 11°C., while in the second series, $T_S - T_A$ was approximately 6°C. The residual air movement in the booth for all experiments was essentially uniform and constant.

Routine of measurement: For each experimental condition at least

three sets of experiments were carried out. Each set consisted of two experiments; and the duration of each experiment was one hour. During this period the weight losses and rectal temperatures were taken at 20-minute intervals, after which followed in order records of metabolism and skin temperature. The total experimental time for each set was two hours and a half. The subject remained nude in a sitting posture for all experiments.

Subjects: Two subjects were chosen, of very different body sizes, shapes and weights. Their general characteristics were as follows:

Subject I { Height: 5 ft., 7 in. (170 cm.)
Weight: 230 lbs. (105 kgm.)

Subject II { Height: 5 ft., 5 in. (165 cm.)
Weight: 105 lbs. (47 kgm.)

Data. The algebraic sum of metabolism, storage and evaporation, H, and the radiation intensity, R' for all the individual experiments are given in table 1 for both subjects I and II. In our experimental code each number represents one set of experiments; the sequent letter "A" represents the experiment for the first hour, "B" for the second, and so on.

In table 2 are given (for each subject) the average of the individual physiological and physical measurements for each of the eleven experimental conditions.

The air movement for all the experiments was approximately 15 feet per minute and the relative humidity approximated 50 per cent.

Results. The data are divided into two series; those for difference between skin and air temperatures of 11°C., and those for differences of 6°C. For the 11° data as plotted for both subjects in figure 1 (a) we see that the observations for the conditions 3, 4, 5, and 6 are co-linear while those for conditions 1 and 2 are non-linear with the others. For the 6° data (fig. 1 b) the observations for conditions 7 and 11 fell outside the linear region formed by conditions 8, 9, and 10. In the discussion to follow later we shall consider in the 11° set that the observations for conditions 3, 4, 5 and 6, and in the 6° set those for conditions 8, 9, and 10, satisfy the Linearity Criterion. For the present it is believed that a visual test of linearity is just as significant as any complicated mathematical or statistical analysis.

Our reasoning so far leads to the conclusion that within the range of Linearity any inherent errors involved in our determinations of metabolism, storage, or evaporation must be either zero or constants. Analysis of the errors which might occur will help us to decide between these two alternatives.

An evaporation error, ϵ_E, may exist when the weight loss (corrected for O_2 gain and CO_2 loss) includes body moisture that has evaporated from

TABLE 1

Summary of data by single experiments

Condition	Subject I			Subject II		
	Experiment code	R'	H	Experiment code	R'	H
		kgm. cal./ hr. m²	kgm. cal./ hr.		kgm. cal./ hr. m²	kgm. cal./ hr.
for $T_S - T_A$ = 11°C.						
1	64-A	−54	88	67-A	−56	92
	64-B	−48	79	67-B	−53	70
	65-A	−53	49	68-A	−53	82
	65-B	−52	80	69-A	−67	114
	66-A	−59	73	69-B	−59	78
	66-B	−54	82	69-C	−54	91
2	67-A	−21	88	70-A	−15	74
	67-B	−17	70	70-B	−12	76
	68-A	−9	80	71-A	−22	81
	68-B	−14	96	71-B	−17	73
	69-A	−19	94	72-A	−19	88
	69-B	−17	56	72-B	−17	55
	70-A	−24	53	73-A	−21	79
	70-B	−19	58	73-B	−16	73
3	71-A	8	85	74-A	7	91
	71-B	12	80	74-B	7	67
	72-A	21	67	75-A	18	64
	72-B	11	67	75-B	18	44
	73-A	19	65	76-A	19	83
	73-B	25	71	76-B	22	27
4	74-A	46	46	77-A	49	45
	74-B	46	33	77-B	46	42
	75-A	41	46	78-A	49	21
	75-B	43	27	78-B	49	20
	76-B	40	29	79-A	48	29
				79-B	50	0
5	77-A	83	−51	80-A	82	3
	77-B	85	−53	80-B	87	−25
	78-A	80	−48	81-A	78	−5
	78-B	88	−29	81-B	86	−5
	79-A	88	−48	82-A	82	−17
	79-B	90	−42	82-B	87	−6
6	80-A	118	−94	83-A	117	−60
	80-B	120	−98	83-B	119	−58
	81-A	119	−103	84-A	120	−68
	81-B	120	−88	84-B	122	−57
	82-A	122	−88	85-A	132	−64
	82-B	118	−105	85-B	132	−58

Condition	Subject I			Subject II		
	Experiment code	R'	H	Experiment code	R'	H
		kgm. cal./ hr. m²	kgm. cal./ hr.		kgm. cal./ hr. m²	kgm. cal./ hr.
for $T_S - T_S$ = 6°C.						
7	94-A	−24	47	98-A	−26	53
	94-B	−25	47	98-B	−22	49
	95-A	−37	65	99-A	−24	50
	95-B	−28	67	99-B	−25	49
	96-A	−34	69	100-A	−32	62
	96-B	−32	87	100-B	−33	66
8	37-B	1	75	36-A	2	56
	40-A	2	46	36-B	7	37
	40-B	5	58	101-A	11	45
	41-B	2	52	101-B	11	42
	97-A	9	46	102-A	8	50
	97-B	10	41	102-B	12	25
9	98-A	32	12	103-A	32	19
	98-B	35	−19	103-B	37	−3
	99-A	34	−9	104-A	36	13
	100-A	35	6	105-A	36	9
	100-B	34	19	105-B	42	0
	101-A	32	13			
	101-B	35	−11			
10	102-A	65	−47	106-A	74	−25
	102-B	70	−55	106-B	72	−28
	103-A	75	−74	107-A	71	−43
	103-B	79	−58	107-B	65	−22
	104-A	69	−76	108-A	76	−43
	104-B	65	−62			
11	105-B	100	−93	109-A	107	−92
	106-A	93	−130	109-B	98	−94
	106-B	95	−126	110-A	102	−83
	107-A	95	−126	110-B	95	−88
	107-B	96	−128	111-A	96	−75
				111-B	92	−91

nearby surfaces other than that of the body itself. ϵ_E represents the deviation from 100 per cent evaporation efficiency. Such an error cannot be constant but must either be zero or vary after a certain critical value of E with the experimental parameter. For the linear portion of an H *vs* R' plot ϵ_E must be zero. For cases where 100 per cent evaporation efficiency does not exist, the true evaporation loss is given by $(E - \epsilon_E)$ since ϵ_E can only be negative with respect to E (i.e., positive with respect to M).

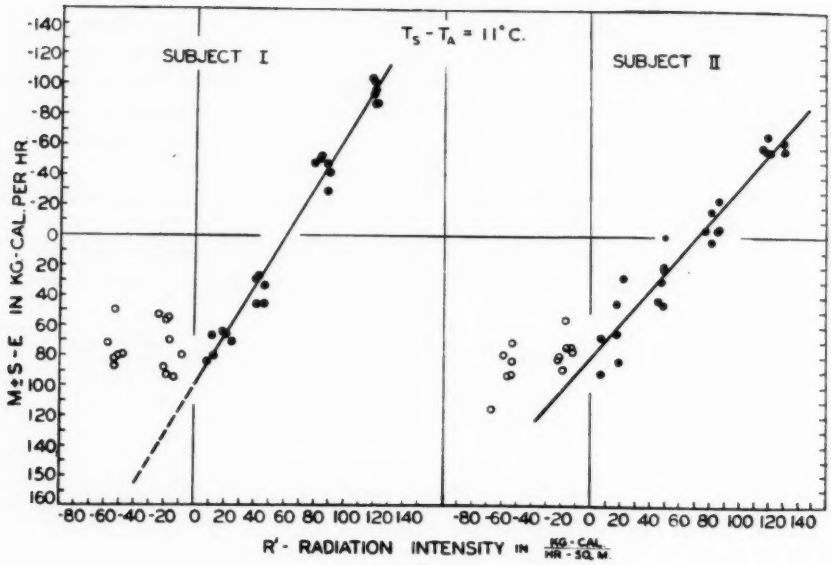
The measured Storage, S, will only represent the true storage when the rate of change of the rectal temperature (or of the oral or any other measured body temperature) is equal to the change in temperature for the whole body. When such a rate of change is not representative of the whole body, this error varies with the parameter R' and linearity would be destroyed. For the linear portion of an H *vs* R' plot ϵ_S must be zero.

TABLE 2

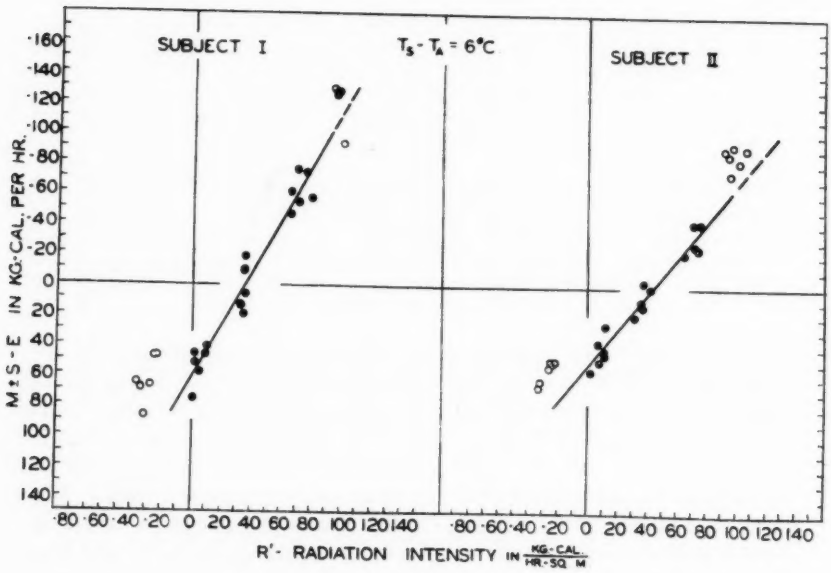
General summary of data

CONDITIONS	T_A	T_W	T_S	$T_S - T_A$	RATE OF WEIGHT LOSS	$T_{B'}$	METABOLISM	T_A	T_W	T_S	$T_S - T_A$	RATE OF WEIGHT LOSS	$T_{B'}$	METABOLISM
	°C.	°C.	°C.	°C.	gr./ min.	°C./hr.	kgm. cal./ hr.	°C.	°C.	°C.	°C.	gr./ min.	°C./hr	kgm. cal./ hr.
1	17.1	19.0	28.9	11.8	0.69	−0.12	87.4	17.1	19.0	29.5	12.4	0.55	−0.47	86.1
2	18.9	28.9	30.9	12.0	1.07	−0.22	91.3	18.7	27.2	30.0	11.6	0.65	−0.32	78.6
3	21.1	35.2	32.3	11.2	1.09	−0.09	97.3	21.4	35.9	33.3	11.9	0.82	−0.21	81.2
4	23.1	42.1	34.3	11.2	1.20	+0.14	87.3	23.3	42.9	34.4	11.1	1.71	−0.09	79.6
5	24.5	49.7	34.8	10.3	4.42	−0.08	98.6	24.1	49.7	35.1	11.0	2.44	+0.14	76.2
6	24.2	56.1	35.3	11.1	5.39	+0.12	98.1	24.0	56.9	35.4	11.4	4.11	−0.05	77.3
7	27.4	28.2	33.5	6.1	1.19	−0.05	100.2	27.4	27.7	32.9	5.5	0.71	−0.03	76.9
8	27.8	34.9	34.1	6.3	1.21	0	93.8	28.5	35.9	34.4	5.9	1.00	+0.13	81.6
9	29.1	40.6	34.7	5.6	2.90	+0.04	103.1	28.7	40.7	34.2	5.5	1.94	+0.07	74.9
10	29.6	46.4	34.4	4.8	4.70	0	99.4	29.6	47.3	34.8	5.2	2.96	+0.13	76.0
11	29.5	52.5	35.3	5.8	6.17	+0.55	94.3	28.9	52.8	35.6	6.7	4.76	+0.21	79.0

So far we have made no mention of the possible error that may be introduced in the measurement of the radiation intensity, R', itself. If a linear relationship should exist between H and our measured R', it is obvious that our measured R' must be directly proportional to the true R'. Further, this same constant of proportionality would enter as a correction factor relating the experimental surface area A_R, to the actual


SUBJECT I
$T_S - T_A = 11°C.$
SUBJECT II
M±S-E IN KG-CAL. PER HR
R'- RADIATION INTENSITY IN KG-CAL. / HR-SQ. M.

A


SUBJECT I
$T_S - T_A = 6°C$
SUBJECT II
M±S-E IN KG-CAL. PER HR.
R'- RADIATION INTENSITY IN KG-CAL. / HR-SQ. M

B

area. The experimental value A_R would then be the "calibrated" radiation surface area for the particular method by which the radiation, R', was measured. In no case would this factor have any effect on conclusions as to the nature of ϵ_S, and ϵ_E mentioned above.

We now see that by plotting the sum H versus the experimental parameter R', holding the convection constant, we get a straight line if, and only if, our physiological measurements of metabolism, storage and evaporation, are actually true readings. Conversely, if by plotting H versus R' we get a straight line, then our physiological measurements are correct. The Linearity Criterion in its present form may now be stated as follows: "All physiological measurements of the heat transfer from the body to the environment will be correct only when the sum of the metabolism, storage and evaporation is a linear function of the radiation intensity from or to the body, provided the convection loss is held constant."

Analysis of factors causing deviations from linearity. Our next problem is to determine the reasons for the deviations which appear in our plot outside the region of linearity. The discussion of this question will be carried out essentially from a physical point of view. The fundamental physiological questions involved will be touched upon only when necessary, since a thorough discussion of this subject will be deferred to a later paper.

The condition of our experiment required that the convection term must be held constant for all temperatures used, by holding both difference between skin and air temperature and air movement constant. The values for $T_S - T_A$ in table 2 show that for both series this difference was held approximately constant, within a tolerance of 2°C., with a random scatter about the mean, throughout the experiments. Since these differences were small and showed no relation to R' they may be eliminated as a cause of any serious variability of the convection term in the two series of experiments. The air movement for all conditions was approximately 15 to 20 feet per minute, as a result of the natural ventilation of the booth. As it varied in a random fashion, air movement cannot cause variability of the convection term in either of the two series. Therefore, the condition of the experiments that $C(T_S - T_A, V)$ be held constant is believed to be satisfactorily validated.

Since a linear portion of an H *vs* R' plot does exist for certain regions of the curve it has been shown in a previous paragraph that our measured R' must be directly proportional to the true R'. Therefore, errors in radiation measurements cannot at any point be the cause for any deviation from linearity.

Since all purely physical causes for non-linearity have thus been eliminated, the deviations from linearity must have a physiological significance. For the non-linear regions some of our measurements are presumably in error. Let us now see how this may have occurred.

For the linear region ϵ_E must be zero. In this region we evidently have a 100 per cent efficiency of evaporation and the measurement of the evaporation loss is correct. In particular, this validates for one of our subjects perspiration losses up to 5 grams per minute, which existed for the hottest condition in the linearity zone. If 100 per cent efficiency exists up to 5 grams per minute, it also holds for those non-linear experiments where the weight loss is lower. Therefore, for all experiments toward the region of low R' in our plots, ϵ_E is negligible and evaporation measurements present no cause for non-linearity. However, in figure 1 (b) where the deviation from the straight line occurs for condition 11, there is a strong experimental probability that there is no longer 100 per cent evaporation. This might be expected, since under such extreme conditions moisture would be lost from the body in other ways than by evaporation, as by transfer to chair and scales. Our measured value of E would be too large, and therefore our observed value for $M \pm S - E$ would be too low. For condition 11 the values of E must be decreased to fall in the linear region; it is probable that for this condition the error ϵ_E may exist and is always positive in sign (that is, opposite to E which is always negative).

For the non-linear portion of our plot toward high R' there is then a probable evaporation error, ϵ_E. Deviations from linearity in any non-linear region may also be affected by storage errors, ϵ_S; and toward low values of R' these seem to be the only possible errors involved. In either of these regions our value H must apparently be increased by a positive error term to become linear. Therefore, storage errors must be positive in either case.

Storage represents the actual change in the energy state of the body and suggests by its very existence that a final equilibrium condition has not been reached. In a state of equilibrium the body temperature, T_B, would remain constant throughout and metabolism and evaporation would have adjusted themselves to compensate for the convection loss and radiation exchange. At the end of a given experimental period the body may be actually either in equilibrium or non-equilibrium. In either case our only method of measurement is the change or constancy of the rectal temperature. For the linear portion of our curve, the rectal changes apparently give the true trend of storage values. We are then able experimentally to say that the body is in equilibrium or, if not, to a certain degree of accuracy how much it is off balance. In this part of the curve it is not necessary for an equilibrium condition to be reached before storage may be measured. For the non-linear region, on the other hand, we have no measurement of storage at all.

As the average rectal changes in table 2 indicate, there is a great deal of variation in regard to the direction of storage. Whatever the variations, it is certain that for the rectal changes to be significant they must

be truly representative of the changes in the actual body temperature itself. The temperature of the body is kept fairly uniform through the transfer of heat by the blood stream throughout its system. The rectal changes when they occur must represent changes in equilibrium of that particular region of the body through which temperature uniformity is thus maintained. When rectal temperatures represent a true measure of storage, this region of uniformity must extend throughout the whole body to the very layers of the skin. For the warmer conditions this is believed, on physiological grounds, to be the cause. Although at present it is not justifiable to make generalizations, it is believed that rising rectal temperatures associated with warm conditions ordinarily yield correct storage measurements while lowering rectal changes may very probably be incorrect as indices of storage when the change is large.

For the condition of low R' we find that our points lie above the straight line as that line is produced from the linear region. This shows that in order that our measurements should be correct the H value must be increased by a positive amount ϵ_S. Thus, S, measured by the rate of rectal change is too small and must be increased by a value ϵ_S to represent the true value. In other words, our experiments suggest that under the conditions in question the body cools off at a greater rate than that indicated by the rectal temperature. This seems highly probable. The rectal temperature is chiefly influenced by the drop in temperature of the trunk but our calculations assume that the whole body drops in temperature at the same rate as the trunk. This cannot be actually the case as it is obvious that the extremities, with their greater surface area per unit volume, may cool faster than the trunk. If this is the case, then the body must actually lose heat at a greater rate than if the body cools uniformly.

The deviation of the observations of condition 11 from linearity have been shown to be caused by either a positive ϵ_R or a positive ϵ_S. Let us consider ϵ_S alone. Table 2 shows that this condition is in general associated with a rising body temperature or negative storage. If ϵ_S must be positive, then it means that the body temperature is not rising at as great a rate as indicated by the rectal temperature, or in other words, the rectal temperature is rising faster than the average body temperature. The data in table 2 do not indicate any abnormality of metabolism in these experiments. Nor is it possible to believe that the flow of blood to the skin (under such a warm condition) is being hampered in such a way that the rise of internal body temperature is greater than that of the outer body layers. All this evidence tends to show that for condition 11, ϵ_S is probably zero and that ϵ_R is the essential error term involved toward high R' values. We have shown for low values of R' (conditions 1, 2, 7) ϵ_S is the fundamental source of error.

In figure 2, the location of each of the experimental conditions to which

the criterion has been applied is plotted with T_W and T_A as ordinate and abscissa. Those conditions which satisfied the criterion are indicated by circles while others are denoted by squares. Arbitrary straight lines have been drawn to separate our experimental regions into three divisions. The deviations in non-linear regions toward large T_W and T_A are associated probably with errors due to evaporation. The non-linear region toward small T_W and T_A are associated probably with errors due to the improper measurement of storage. The central zone represents the region where there exists greatest probability that physiological measurements of the energy changes of the body are valid.

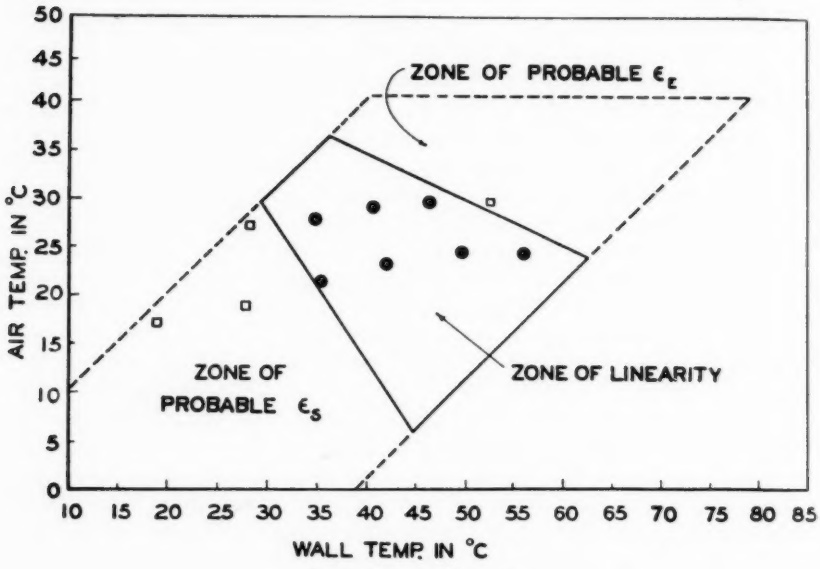


Fig. 2. Zone of linearity.

One notices that the relative humidity enters nowhere into our theoretical set-up, and the question arises, how far relative humidity may affect the application of the Linearity Criterion and the boundaries of the Zone of Linearity. For experiments toward the colder region, where the criterion fails, the evaporation loss is very low and is unlikely to be influenced by changes in relative humidity. As humidity affects only the evaporation loss from the body it is believed that the boundary between the linear zone and the non-linear zone of probable storage error remains very much the same for all humidities. The boundary between the linear zone and the zone of probable evaporation error lies in a region where such losses

normally are very large. It is therefore reasonable to suppose that this boundary will be substantially affected by humidity changes, with a tendency to narrow the linear region of true measurement as the humidity increases.

For the present, the conclusions derived from our plots apply only to the special case where the humidity is approximately 50 per cent, but the significance of this method of analysis must not be overlooked in the study of the effect of relative humidity on the individual partition factors themselves.

It has been pointed out above that our values for radiation intensity and effective radiation area are so interrelated that no radiation errors exist which affect the linearity relationship. That our measured R' is not only proportional to, but probably identical with, the true R', is suggested by the following line of reasoning.

For the linear region equation (6) may be written

$$H + A_R R' = C. \tag{7}$$

Let A_R and R' in (7) represent the true value of area and radiation intensity. Then if R'_m is our measured radiation intensity, a linear relationship between H and R'_m requires that

$$H + A_R k R'_m = C, \tag{8}$$

where

$$R = kR'_m. \tag{9}$$

The constant k as defined by (9) is essentially the potential calibration error factor in our measurement of R'_m. The product $(A_R k)$ is the actual value of the slope of our linear plots. Our calibration of R' is accurate when our measured wall temperature is equal to our true wall temperature and when our measured skin temperature is equal to our true skin temperature. However, by (9) when the measured skin temperature is equal to the measured wall temperature, the radiation exchange is zero and the true wall temperature is equal to the true skin temperature. Since the wall temperature under such a condition is considerably above the air temperature and measured independently of the skin temperature, it seems that the elimination of radiation exchange is sufficient to justify the assumption that the calibration factor k is unity. Therefore, the slopes of the linear portions of figure 1 (a) and 1 (b) must represent the true surface area of the two subjects if the $T_S - T_A$ and V of the convection function is held absolutely constant. Corrections due to such fluctuations in the convection function will be applied in a later paper to obtain this value with greater precision.

CONCLUSIONS

In a dynamical treatment of heat balance between metabolism and environment, the physiologist is confronted with a twofold problem. First, a method must be found of completely separating the five elements of partition so that each element has an individual significance in relation to certain easily derived physical and physiological measurements. Secondly, the many interrelationships between these elements must be examined for various physical conditions. Earlier studies in this field have depended on attempts to observe either the relationship between a given individual element of partition and its associated physical and physiological measurements or the relationship between two such elements, holding the other three relatively constant. A more general approach is presented in this paper. By means of the First Law of Thermodynamics and certain physiological and physical assumptions, not only the relationship between each element of partition and its basic variable, but also the interrelationship between the five elements themselves are formulated at the start. This partition, theoretically complete, is next analyzed in such a way that a linear relationship between certain groups of basic measurements will hold only when our physiological and physical assumptions are valid. Where this linearity criterion is satisfied, each element of partition may be studied in terms of its fundamental measure. Furthermore, the interrelationship between all the elements themselves is completely presented both qualitatively and quantitatively. Where this criterion fails, there is a probability that an error exists in one of our basic measurements. It has been shown—in our special case—that for such deviations toward the colder conditions there is an error in the storage measurement, while for the hotter conditions, there is an error in the evaporation measurement.

REFERENCE

WINSLOW, C.-E. A., L. P. HERRINGTON AND A. P. GAGGE. This Journal **117**: 641, 1936.

THE DETERMINATION OF RADIATION AND CONVECTION EXCHANGES BY PARTITIONAL CALORIMETRY

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Contribution No. 12 from the John B. Pierce Laboratory of Hygiene, New Haven, Connecticut

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Objectives and general results. In a previous contribution from this Laboratory (Winslow, Herrington and Gagge, 1936) we have described a new method of analyzing by partitional calorimetry the thermal behavior of the human body under widely varying conditions of radiation and convection, and have presented a mathematical criterion (Linearity Criterion) (Gagge, 1936) by which the reliability of the data obtained may be determined. The present paper deals with the actual interchange of heat between the body and its environment, partitioned between radiation and convection, over a certain range of conditions.

The experimental methods employed and the analytical procedures applied to the results are fully described in the papers cited. It need only be repeated here that the work was performed with two experimental subjects of widely different body builds;[1] and that these subjects were observed nude and seated in a chair placed on a balance for weighing evaporation loss. The chair was located in the center of a copper booth arranged to reflect radiant heat of any desired intensity to its interior, and air temperature was controlled with a high degree of accuracy. The period of each experiment was one hour. Data considered in the present paper all fall within the experimental zone validated by the Linearity Criterion —that is, the relation between the algebraic sum of metabolism, storage and evaporation loss, and the computed radiation loss per unit area of body surface is linear (when convection loss is held constant). This relationship, as we believe, guarantees that no errors of serious magnitude enter into our observations or analytical procedures. Altogether, 98 experiments with subject I and 75 experiments with subject II are here included.

The experimental data, thus accepted as valid, are summarized in table 1. Observations made under colder conditions, where the linear relationship fails as a result of imperfect evaluation of storage, will be dis-

[1] Subject I: height 5 feet, 7 inches; average weight, 230 pounds. Subject II: height 5 feet, 5 inches; average weight, 105 pounds.

TABLE 1

General summary of data

SUBJECT	GROUP OF EXPERIMENTS	CONDITIONS	NUMBER OF EXPERIMENTS	AIR MOVEMENT	RELATIVE HUMIDITY	T_A	T_W	T_S	WEIGHT LOSS	CHANGE IN RECTAL TEMPERATURE	M	S	E	R	C
				ft./min.	*per cent*	°C.	°C.	°C.	*gr./min.*	°C./hr.	*kgm.-cal. per hr.*	*kgm.-cal. per hr.*	*kgm.-cal. per hr.*	*kgm.-cal. per hr.*	*kgm.-cal. per hr.*
I	A	3	6	15	51	21.1	35.2	32.3	1.09	−0.09	97	8	−35	25	−95
		4	5	15	49	23.1	42.1	34.3	1.20	+0.14	87	−12	−40	68	−103
		5	6	15	44	24.5	49.7	34.8	4.42	−0.08	99	7	−150	135	−91
		6	6	15	44	24.2	56.1	35.3	5.39	+0.12	98	−10	−184	189	−93
		12	14	15	52	17.1	54.4	34.2	3.71	−0.05	98	3	−125	181	−157
	B	8	6	20	50	27.8	34.9	34.1	1.21	0	94	0	−41	8	−61
		9	7	20	46	29.1	40.6	34.7	2.90	+0.04	103	−3	−98	54	−56
		10	6	20	42	29.6	46.4	34.4	4.70	0	99	0	−160	111	−50
	C	13	10	47	39	22.5	44.6	33.7	1.33	−0.12	101	10	−46	97	−162
	D	14	8	70	39	21.4	49.6	32.9	1.46	−0.12	103	10	−48	148	−213
	E	15	10		45	32.2	35.4	34.7	2.52	+0.10	102	−8	−85	5	−14
		16	14		47	31.3	35.2	34.6	1.92	+0.11	102	−9	−64	7	−36
II	A	3	6	15	51	21.4	35.9	33.3	0.82	−0.21	81	9	−27	17	−80
		4	6	15	49	23.3	42.9	34.4	1.17	−0.09	80	4	−57	53	−80
		5	6	15	44	24.1	49.7	35.1	2.44	+0.14	76	−6	−79	92	−83
		6	6	15	44	24.0	56.9	35.4	4.11	−0.05	77	2	−140	140	−79
		12	13	15	51	16.6	54.3	34.8	2.01	+0.19	72	−8	−68	127	−123
	B	8	6	20	50	28.5	35.9	34.4	1.00	+0.13	82	−6	−33	9	−52
		9	5	20	46	28.7	40.7	34.2	1.94	+0.07	75	−3	−65	40	−47
		10	5	20	42	29.6	47.3	34.8	2.96	+0.13	76	−6	−101	79	−48
	E	15	8		48	33.1	35.4	34.9	2.07	+0.05	79	−2	−70	3	−10
		16	14		48	31.4	35.2	34.8	1.96	0	77	0	−65	5	−17

Subject I was the pyknic, subject II the leptosomic subject.

The groups of experiments (A-E) are based on extent of air movement. In group E the air movement was not recorded.

The conditions are based on the air and wall temperatures maintained. Conditions 3 to 6 and 8 to 10 were used in a previous paper (Gagge, 1936) to illustrate the application of the Linearity Criterion.

Air movement is expressed in feet per minute.

Relative humidity is in terms of per cent saturation.

T_A, T_W and T_S are the temperatures of air, wall and body surface in Centigrade degrees. In the case of the wall the figure given represents an equivalent mean black-body radiant temperature.

Weight Loss is in grams per minute.

The change in rectal temperature is in degrees Centigrade per hour.

M (metabolism), S (storage), E (evaporation), R (radiation), and C (convection), are expressed in terms of kilogram-calories per hour. A negative sign indicates a storage (warming of the body) or a loss of energy to the environment. The absence of a sign indicates the cooling of the body or a gain of energy from the environment.

cussed in a subsequent analysis of that problem. Observations made under conditions warmer than those included in the linearity zone cannot be used at all since in this region the recorded weight loss does not give a true measure of evaporative cooling on account of the loss of perspiration from the body before evaporation.

The data in the upper part of table 1 refer to the stout subject (I) and each row represents the average of from 5 to 14 experiments conducted under approximately the same environmental conditions. The experiments have been grouped first according to air movement. In group A the velocity of the air surrounding the subject was approximately 15 feet per minute; in group B, 20 feet; in group C, 47 feet; in group D, 70 feet. Group E included two early sets of experiments in which the air velocity was not recorded; but, from the fan speeds and other engineering conditions, the velocity was probably between 15 and 25 feet per minute. Conditions 3, 4, 5 and 6 of group A and conditions 13 (group C) and 14 (group D) all had approximately the same value for ΔT (the difference between skin temperature and air temperature), of about 11°C. Under conditions 12 of group A, ΔT was higher (over 16°C.); under conditions 8, 9, and 10 of group B it was lower (5°C.); and under conditions 15 and 16 of group E it was still lower (1°–3°C.). Where both air movement and ΔT are alike (as in conditions 3, 4, 5, and 6, and in conditions 8, 9, and 10, respectively) the conditions are separated on the basis of differing wall temperatures.

The lower part of table 1 shows similar data for the slender subject (II). In this case, conditions 13 and 14 with high air velocities were not duplicated. All analyses leading to our conclusions were, of course, made on the basis of the 173 individual experiments and the mean values in table 1 are presented merely to give a picture of the scope of work and the general trend of results.

All the values in table 1 represent the means of direct experimental observations, insofar as air temperature, wall temperature, skin temperature and relative humidity are concerned. Data for metabolism, storage and evaporation also depend on direct observations of metabolism, change in body temperature, and loss of weight, respectively, converted into corresponding values in kilogram-calories by the usual method. Data for radiation and convection involve the determination of constants which will be discussed in detail in succeeding paragraphs. A few comments on the general significance of the values in table 1 may not, however, be out of place.

It will be noted that the air temperatures to which our subjects were exposed ranged (by group averages) from 16.6°C. to 33.1°C., with effective black-body radiation wall temperatures ranging from 34.9°C. to 56.9°C. Relative humidity means for the various groups ranged from 39 to 52 per cent of saturation.

Mean skin temperatures ranged (for groups of experiments) from 32.2°C. for the heavily-built subject (I) to 35.4°C. for the thin subject (II). In general, the skin temperature of the thin man was about three-tenths of a degree higher than that of the fat man. On the other hand, the total metabolism[2] of the fat man (I) was, of course, much higher (87–103 kgm. cal. per hour) than that of the thin man (72–82 kgm. cal. per hour). Storage changes were slight and variable. Heat loss by evaporation, it will be noted, rises in all cases with increase in either wall or air temperatures but is almost always higher for the fat subject (I), ranging from 35 to 184 kilogram-calories per hour, while for the thin subject (II) it ranges from 27 to 140 kilogram-calories per hour.

Radiation effects in relation to body surface. So far as gain or loss of heat by radiation is concerned (in the experiments here reported it was always gain) our conclusions are based on the mean effective black-body temperature of the surrounding walls and of the body surface, measured by appropriate thermopile methods. These methods have been described by Winslow, Herrington and Gagge (1936) and their general accuracy validated by the analysis of Gagge (1936). From such data it is, of course, possible to compute heat interchange per unit of surface area directly from the Stefan equation

$$R' = k_R(T_W^4 - T_S^4), \tag{1}$$

where k_R is the universal radiation constant and equal to 4.92×10^{-8} kg. cal. per sq. meter per hour, T_W, the equivalent black-body wall temperature in degrees Absolute, and T_S, the mean skin temperature in the same terms. The reasons for considering constants referring to angle factor and emissivity as unity have been presented in a previous paper (Gagge, 1936).

A complete partition of thermal interchange between the body and its environment necessitated the conversion of such figures for radiation transfer per unit area into terms of total transfer of the body as a whole. This obviously required a knowledge of that part of the total body surface actually effective in radiation. This surface cannot be computed directly from the DuBois, or any similar, formula. It will always be less than the total surface of the body, in proportion as legs, arms and other parts radiate to each other, and not to surrounding environmental surfaces. The effective radiation surface will furthermore vary somewhat widely with the

[2] Subject I: Range of metabolism per kilogram of body weight, 0.84 to 0.99 kgm. cal. per hour. Range of metabolism per square meter of surface area, 40.9 to 48.4 cal. per hour.

Subject II: Range of metabolism per kilogram of body weight, 1.51 to 1.72 kgm. cal. per hour. Range of metabolism per square meter of surface area, 48.3 to 55.0 cal. per hour.

posture of the individual; and it must be determined experimentally for a given position of a person of a given body build.

Fortunately, the set-up of our experiments makes it possible to determine this effective radiation surface with relative ease. In a previous paper of this series, Gagge (1936) has presented graphs indicating the relationship between the sum of the heat production and heat interchange, due to metabolism, storage, and evaporation, and the intensity of radiation between the body and its environment per unit area of body surface. Within those limits indicated by the Linearity Criterion (and with the difference between skin and air temperature held approximately the same so that convection loss is constant) we obtain a straight line when we plot H (the algebraic sum of metabolism, storage and evaporation) against R' (the radiation interchange per unit area). Since the ordinate in such a graph represents a total value of H for the body as a whole, while the abscissa, R', is an intensity factor per unit of surface area, it is obvious that the slope of the line in such a graph must correspond to the effective radiation area which we are seeking.

In the previous paper it has been shown that the partition equation may be written

$$H + A_R R' - C = 0, \tag{2}$$

where

$$H = M \pm S - E.$$

C was held approximately constant by holding the value of ΔT constant in each series of experiments. The value of A_R, found by a "simple regression equation" between H and R', gives a measurement of the surface area uncorrected for any fluctuation of the ΔT in the convection term C. Such fluctuations may be compensated by assuming that C is proportional to ΔT. Equation (2) then becomes

$$H = -A_R R' + k\Delta T, \tag{3}$$

where we have two independent variables instead of one as in equation (2). The true value for A_R, is now given by a "multiple regression equation" between H on the one hand and R and ΔT on the other. The values for A_R and k found by such a method are given in the following table.

Regression constants for equation (3)

	ΔT = 11°C.			ΔT = 6°C.		
	A_R	k	Number of experiments	A_R	k	Number of experiments
Subject I	1.60	8.81	23	1.55	9.88	19
Subject II	1.11	7.06	24	1.10	8.77	16

Weighting the values of A_R by the number of experiments used in determining each value, we find for

$$\text{Subject I} \quad A_R = 1.58 \text{ m}^2,$$

and

$$\text{Subject II} \quad A_R = 1.11 \text{ m}^2.$$

The effective radiation surface area (with the subject reclining in a chair) is found to be 1.58 square meters for subject I and 1.11 square meters for subject II. It may be of interest to compare these figures for effective radiation surface area with those which would be derived from the DuBois formula for total surface area.

	TOTAL AREA, DUBOIS	EFFECTIVE RADIATION AREA	PER CENT TOTAL AREA EFFECTIVE
	sq. m.	*sq. m.*	
Subject I	2.13	1.58	74
Subject II	1.49	1.11	75

The correspondence seems very satisfactory, particularly when we note that Bohnenkamp (1931) found an effective radiation area for his subjects, in a standing position, which was about 85 per cent of the DuBois total. This is exactly the sort of relationship one would expect, since effective radiation surface should be less in the standing position than the DuBois values for total surface, and should be still further reduced in the sitting position.

These values for the effective radiation surface (1.58 sq. m. for subject I and 1.11 sq. m. for subject II) were therefore employed in computing such total radiation gains as are summarized in the "Radiation" columns of table 1.

Heat loss by convection. The second major purpose of this present study was to determine a suitable function, in terms of ΔT (the difference between skin temperature and air temperature) and V (the air velocity), that describes explicitly the convection loss from the human body. There are two general forms in the literature which describe this function. The first may be written

$$C = A(\Delta T)^a + B(\Delta T)\, V^b, \tag{4}$$

where the first term represents the loss due to "free" convection and the second the added loss caused by "forced" air movement. The letters A, a, B, b, are arbitrary constants.

In our previous studies of heat losses from a heated blackened cylinder

with rounded ends (Winslow, Gagge, Greenburg, Moriyama and Rodee, 1935) the first exponential constant, a, was found to be 0.96 and the second, b, 1.27. Other observers have reported a value for the exponent a greater than unity for bodies of various shapes (Glazebrooke, 1922). For the second exponent, b, Fishenden (1925) reports a value (1.37) for a relatively small black body. Within a limited range of low air velocities such as occur under normal conditions of ventilation, we may assume for purposes of preliminary analysis that both the exponential factors equal unity. If such is the case the general equation described in (4) may be simplified to

$$C = (A + B\,V)\,\Delta T. \tag{5}$$

The validity of such a functional type as (5) would be indicated by an experimentally determined linear relation between $C/\Delta T$ and V.

The second general functional form used extensively in the literature to describe the convection loss is

$$C = A\,\sqrt{V}\,\Delta T. \tag{6}$$

Here there is only one arbitrary constant, A. This form obviously cannot hold true as the air movement, V, reaches zero. Under normal conditions of life, and in particular, under conditions possible with our experimental set-up, the free air movement is rarely less than 15 feet per minute. In any case the validity of the functional type (6) would be expressed experimentally by a linear relation between $C/\Delta T$ and $\sqrt{V}$. Bedford and Warner (1935) have verified (6) down to approximately 15 feet per minute for their Globe Thermometer.

Our problem is to determine what equational type describes with least error the observed convection losses from the human body and what are the constants involved. From equation (2) above we see that the algebraic sum of the metabolism, storage, evaporation and total radiation must equal the convection loss. In the preceding section the method of measuring total radiation has been described. Therefore, we are now in a position to determine the convection loss for any experiment where these four quantities are given. In order to test whether (5) or (6) is our proper functional type it is necessary to carry out a series of experiments in which the velocity, V, is varied independently of ΔT, and to test the linearity of $C/\Delta T$ with V or $\sqrt{V}$. For this purpose it was therefore necessary to add to our experimental set-up methods of varying and measuring the air movement about the body.

The air movement about the subject under our previous experimental conditions was of a turbulent variety. For those conditions in the booth where no extra fans were used (groups A and B) the air movement about the seated subject was generally in an upward direction. Its actual rate

was largely conditioned by the amount of air change in the chamber outside the booth which was slightly higher in group B than in group A (on account of the higher air temperature which had to be maintained). For experiments in groups C and D this turbulent air motion was increased by setting three pairs[3] of six-inch fans near the top of the booth on the three side panels, to the left of the openings admitting radiation from the radiant heaters (see fig. 1, Winslow et al., 1936). The air stream from these fans was directed downward toward the floor of the booth, missing the body but reflecting upward from the floor so as to produce turbulent movement in the same general direction as that taken by the air in the fanless condition. The resulting air movement was measured by a hot-wire anemometer held six inches from the body over the fifteen positions corresponding to the points at which the skin temperatures were taken. The recorded measurements were then averaged for four representative areas of the body; the head, the upper extremities, the trunk, and the

TABLE 2

Distribution of air velocities over various areas of the body

WEIGHT FACTOR	HEAD (7)	UPPER EXTREMITIES (21)	TRUNK (31)	LOWER EXTREMITIES (41)	WEIGHTED MEAN (100)
Group A	12*	10*	12*	20	15
Group B	20	20	20	21	20
Group C	25	32	49	56	47
Group D	58	54	69	82	70

* Probable values—our hot-wire anemometer is sensitive only to 6 feet per minute about this region and reproducible results are very difficult to obtain.

lower extremities. The distribution of these velocities for the four groups of conditions (A, B, C, and D) of table 1 are presented in table 2. The values in the column headed "Weighted Mean" represent the average of the readings for head, upper extremities, trunk and lower extremities, weighted according to the probable percentage of body surface they represent. The weighting factor is indicated at the head of each column. In general, the air movement was greater about the lower extremities, while, due to the subject's sitting position, the movement was lower for the trunk and still lower (with high air velocities) for the upper extremities and head on account of the relatively shielded position of these parts.

The data for our convection analysis have been completely summarized in table 1. The conditions 3, 4, 5, 6, 8, 9, and 10 have been shown in the previous paper to lie within the linearity zone. For condition 12,

[3] In group C one fan of each pair was operated while in group D both fans of each pair were used.

the T_A and T_W also presumably lie in the linearity zone. In conditions 13 and 14 the forced air velocities are introduced for a ΔT of 11°C. At the same time the radiation term has been correspondingly increased to compensate for the additional convection loss. In this way an effort was made to keep our conditions in a range where the Linearity Criterion

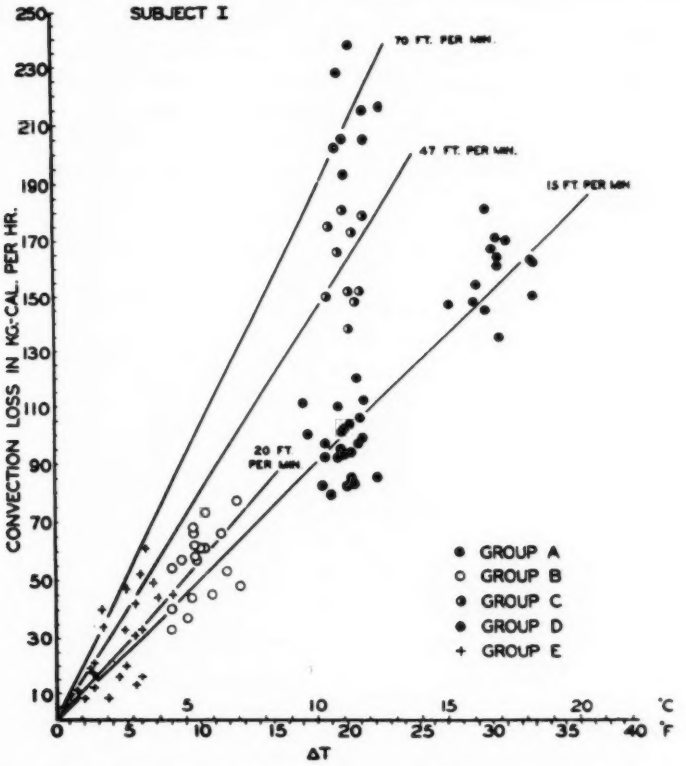


Fig. 1. Relation of heat loss by convection to difference between skin and air temperature at varying air velocities. (Subject I.) Group A-15 feet per minute; group B-20 feet per minute; group C-47 feet per minute; group D-70 feet per minute; group E-unrecorded.

should hold. The logic of this assumption will be borne out by our results which are to follow. Conditions 15 and 16 also lie in the linearity zone in regions where ΔT approaches zero. The air movement here was not recorded. In the last column of table 1 the convection term is found by difference and represents the convection loss for the associated ΔT and air movement, V.

Each of the individual experiments of the five groups in table 1 for subjects I and II has been completely partitioned for metabolism, storage, evaporation, radiation and convection. The total radiation was calculated for each subject from the radiation surface areas given above. In figure 1 and figure 2 the value of his calculated convection term, C, is

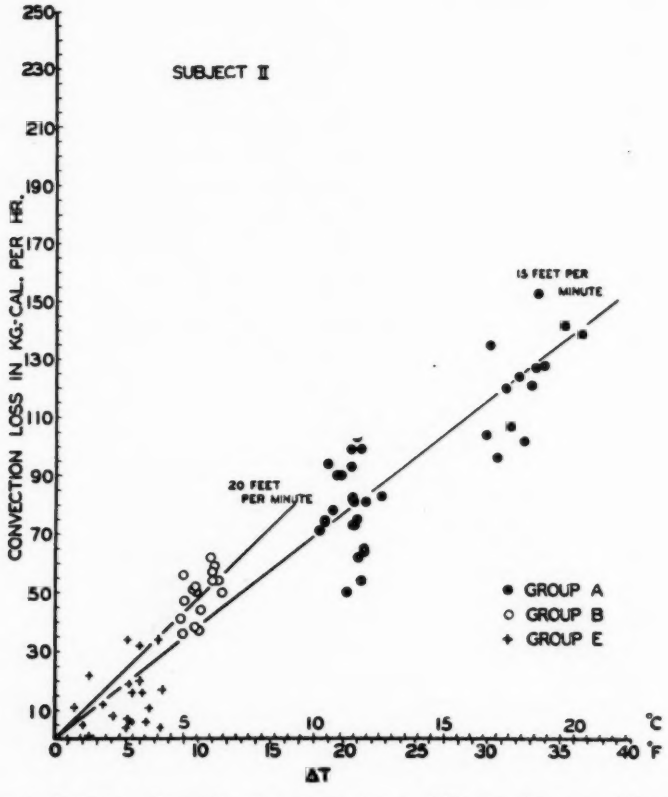


Fig. 2. Relation of heat loss by convection to difference between skin and air temperature at varying air velocities. (Subject II.) Group A-15 feet per minute; Group B-20 feet per minute; group E-unrecorded.

plotted on the ordinate and the ΔT on the abscissa for subjects I and II respectively.

Each point in figure 1 represents a single experiment and each type of symbol for a point represents a group of experiments defined by an air velocity (group A, 15 feet per minute; group B, 20 feet per minute; group

C, 47 feet per minute; group D, 70 feet per minute, and group E, an unknown air velocity of about 25 feet per minute). Trend lines have been drawn from the origin through the clusters of points corresponding to each of the known air velocities.

In group C and in group D, it will be noted that the points lie in a single cluster, since each of these groups included only a single atmospheric condition. In group B, although there were three conditions included, the ΔT in all three was nearly the same, and the points again form a single cluster. In group A, however, there were four conditions with a ΔT of about 11°C., and one condition (14) with a ΔT of about 16°C. The points for this latter condition form a cluster widely separated from those for the conditions with lower ΔT. These two sets of observations of group A (with the same air velocity) are colinear with the origin as would be expected. The points for groups B, C, and D when plotted on lines which extend toward the origin exhibit progressively greater slopes, which

TABLE 3

Relation between air velocity and $C/\Delta T$

SUBJECT	GROUP	AIR VELOCITY	MEAN $C/\Delta T$	PROBABLE ERROR
I	A	15	9.0	±0.6
	B	20	10.1	±1.3
	C	47	14.4	±1.4
	D	70	18.5	±1.3
II	A	15	7.0	±0.8
	B	20	8.7	±0.8

is to be expected according to (5) or (6). The points of group E also harmonize with the conclusion that the value of the convection loss approximates zero as ΔT approaches zero.

Figure 2 shows a similar plot for subject II. The arrangement of the plot and the symbols are the same; but in this instance only two observed air velocities (15 and 20 feet per minute) are available.

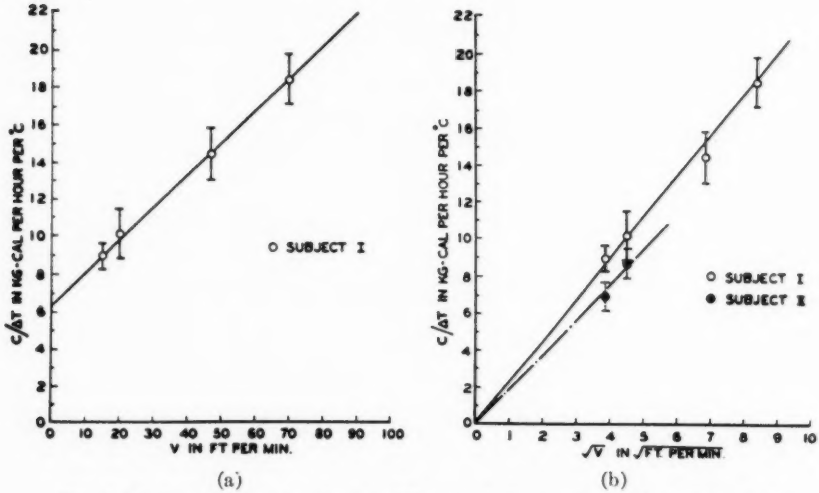
It is at once obvious that the slope of the lines in these figures toward the origin is directly related to air velocity and is measured by the ratio $C/\Delta T$. This ratio we have seen from (5) or (6) is a function only of the air velocity, V. If, for each experiment of every group, the ratio of $C/\Delta T$ is evaluated and averaged, we should be in a position to calculate the values of A and B, provided the probable velocity for each of the groups is correctly known. The values of $C/\Delta T$ so found, are presented in table 3.

We are now in a position to compare the two functional types (5) and (6). For subject I in figure 3(a) we have plotted on the ordinate $C/\Delta T$

$\left(\frac{\text{kgm. cal}}{\text{hr. °C}}\right)$ vs V feet per minute) on the abscissa. The probable errors in $C/\Delta T$ are also entered extending up and down from their mean ordinate value. If the points in figure 3(a) are weighted according to the ratio of the mean $C/\Delta T$ to its probable error one obtains from the mean regression line by least squares the convection equation

$$C = (6.51 + 0.17V)\Delta T \frac{\text{kgm. cal.}}{\text{hr.}} \text{ for °C. and feet per minute,} \qquad (7)$$

whereby the constants, A and B, are evaluated. For subject II no observations were made for high air velocities which would allow the determination of the constants in (5) with accuracy.



(a) (b)

Fig. 3. Ratio of convection loss to difference between skin and air temperature plotted against air velocity (a) and square root of air velocity (b).

In figure 3(b) for both subjects we have plotted $C/\Delta T$ on the ordinate *vs* $\sqrt{V}$ on the abscissa with the probable error indicated as above. The data for subject I fits the relation

$$C = 2.30\sqrt{V}\,\Delta T \frac{\text{kgm. cal.}}{\text{hr.}} \text{ °C. and feet per minute,} \qquad (8)$$

and for subject II

$$C = 1.87\sqrt{V}\,\Delta T \frac{\text{kgm. cal.}}{\text{hr.}} \text{ °C. and feet per minute.} \qquad (9)$$

For subject I in figure 3(a) and figure 3(b) we see that $C/\Delta T$ is linear with either V or $\sqrt{V}$ for the range of air velocities from 15 to 70 feet per minute. We conclude therefore that it makes little practical difference which functional type, (5) or (6), is used, as their deviations from each other are negligible in the range of air movement under consideration. What the relationship may be at very high air velocities is a physical problem of considerable interest, but the answer to this problem is still uncertain. If the actual relation in our experimental range forms a part of a parabola, that part of the parabola involved may be described by the equation of a straight line within the limits of experimental errors. We can now obtain convection values for both subjects based on equation (6)

TABLE 4

Convection losses in kilogram-calories per hour

GROUP	CONDITION	SUBJECT I			SUBJECT II	
		By observation	Computed from equation (8)	Computed from equation (9)	By observation	Computed from equation (9)
A	3	95	95	102	80	86
	4	103	95	102	80	80
	5	91	87	93	83	80
	6	93	94	101	79	83
	12	157	145	155	123	132
B	8	61	62	62	52	48
	9	56	55	56	47	46
	10	50	47	47	48	44
C	13	162	168	162		
D	14	213	211	213		

and for subject I based on equation (5) for comparison with convection losses, obtained experimentally as presented in table 1. This comparison is made in table 4. The probable error of estimate between the computed and observed values of table 4 for either formula is approximately $\pm$ 3 kilogram-calories per hour. Small inaccuracies in the measurement of air velocity will naturally produce considerable deviations, particularly under condition 12 where they are multiplied by large values of ΔT. As a matter of fact, a change in the estimated air velocity used in our computations of from one to three feet per minute would produce practically a perfect concordance with observed results.

The preceding analysis, of course, refers to the figures in table 1, each of which represents the mean of 5 to 14 different experiments for a single

condition. Applying the same test to the individual experiments themselves (74 for subject I and 47 for subject II), we find a probable error of estimate (of the computed value for either formula compared with the observed value) of ±9 kilogram-calories per hour. Since the observed terms for convection were found by difference, this same probable error of estimate applies also to the observed values of H (the algebraic sum of metabolism, storage, and evaporation). Therefore, the probable error of estimate of the sum of metabolism, storage and evaporation by our method of partitional calorimetry is ±9 kilogram-calories per hour for a single experiment. If we refer the probable error of estimate to the standard of metabolism, we may conclude that a single observation in a partition of heat loss is accurate within, at the most, 10 per cent of the metabolism while an average of at least five duplicate experiments gives results reliable within 4 per cent.

SUMMARY AND CONCLUSIONS

1. The method of partitional calorimetry, in common with all methods of calorimetry, is based on one fundamental physical law, the First Law of Thermodynamics. A mathematical criterion (Linearity Criterion) has been used to test the validity of our measurements and, within certain ranges of air and wall temperatures, these were found to be reliable. Outside of such limits it has been demonstrated that deviations occur which are obviously explicable on the reasonable assumption that, at one extreme, our storage measurement and, at the other extreme, our evaporation measurement is in error.

2. Experiments lying in the region properly defined by the Linearity Criterion were chosen for the present paper from which to complete the partition. A value equal to the algebraic sum of metabolism, storage, and evaporation was separated by physical and analytic methods into radiation and convection, making the partition complete. For the Linearity Zone, this partition may be completed without dependence upon any unproved assumptions.

3. One of the by-products of the separation of radiation and convection in the physical analysis mentioned in the preceding paragraph is the evaluation of the effective radiation surface area of the body. This area, we believe, represents the true effective radiation surface area of a given human body in a given position. Our values of A_R for the two subjects have the same ratio as their true areas, although their absolute values depend on the correspondence between our computed values for T_S and T_W and the actual mean effective radiation properties of skin and wall. That no serious errors are involved is suggested by the reasonable correspondence between our effective surface areas and the value derived for effective radiation area by Bohnenkamp (1931) and for total surface area

by DuBois (1927). Our effective radiation area is 75 per cent of the DuBois total surface area.

4. For partition of heat loss due to convection, two equational types have been utilized and both found to fit the data within a limited range of air movment (namely, 15 to 70 feet per minute). No assumptions are necessary concerning the convection equations unless these equations are applied beyond this range of calibrated air movement.

5. A comparison of the convection values as computed from these equations with the convection losses obtained by difference (from our data on metabolism, storage, evaporation and radiation) furnishes an experimental test of our entire process of analysis and of the accuracy of all observations. The coincidence between these independently computed values (as given in table 4) has a probable error of less than 3 kilogram-calories per hour or less than 4 per cent of the hourly metabolism.

6. Experiments so far conducted—while primarily designed for the evaluation of our basic equations—have already yielded concrete results of some physiological interest. They have all been made under conditions which involve exposure of the unclothed body to positive radiation from surfaces at temperatures varying from 35°C. to 56°C., with corresponding air temperatures varying from 33°C. to 17°C. The degree of positive radiation employed has accounted for a gain of heat to the body varying from 3 to 189 kilogram-calories per hour. The varying air temperatures have yielded convection losses ranging from 10 to 213 kilogram-calories per hour; and the energy balance has been completed by an evaporation loss varying from 27 to 184 kilogram-calories per hour.

7. In future studies we propose to generalize our conception of effective radiation surface by a study of the behavior of a number of individuals of varying body builds; and also to analyze further the problem of heat storage by examination of deviations from the linearity curves in the lower ranges of heat loss.

We believe, however, that the methods used have already justified themselves by making available a useful new procedure for measuring thermal interchange between the human body and its environment. This method we have designated as Partitional Calorimetry because it determines all essential data at a given moment (and not over a long period of integrated reactions); and also because it differentiates between effects due to convection and those due to radiation.

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THE INFLUENCE OF THE PYLORUS ON THE SECRETION OF ACID BY THE FUNDUS

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Whether or not the pylorus exerts a specific stimulating effect on the acid secreting mechanism of the fundus and thus plays a rôle in the intragastric chemical phase of acid secretion is still an unsettled question. Studies on fundic pouches have given contrary results so that some investigators claim that acid secretion in the pouch is lowered by pylorectomy (1, 2, 3) while others have found no change (4, 5, 12).

Removal of the pylorus with anastomosis of the fundus to the jejunum (partial gastrectomy) has been found by most recent investigators to cause a definite lowering of acidity (2–11.) Priestley and Mann (12), however, failed to find any change in acidity. Regarding the cause of the lowered acidity usually found there is again lack of agreement; some believe that it is the result of dilution and neutralization by the increased amount of duodenal secretions which enter the stomach (4, 5, 12) while others attribute it to removal of the specific influence of the pylorus (1, 2, 6, 7, 8, 9, 11). It is possible that these divergent findings and interpretations are due either to faulty and inadequate methods of gastric analysis or to improper experimental conditions. The following general statements will illustrate the nature of these errors and their influence on the results:

1. To our knowledge no one has ever employed methods which allowed a strict comparison to be made between the lowering of acidity and the increase in duodenal secretions entering the stomach after partial gastrectomy. Hence the statement that the lowering is due entirely to the duodenal secretions is unproven. 2. A meat and water test meal fed by mouth has frequently been used. This may evoke sufficient psychic secretion to overshadow an otherwise definitely lowered acidity (2). 3. When a meat and water test meal is used, digestion of the meat causes a marked increase in its alkali combining power which may be so marked that it will obscure specific changes in acid secretion; in other words, there is danger of titrating changes in the test meal due to digestion rather than specific changes in acid secretion. 4. Following partial gastrectomy with a Polya anastomosis, the test meal usually leaves the stomach more rapidly,

hence there will be less fluid from the test meal to dilute the acid secretion. Thus if the acid secretion was reduced in amount by one half, but if only half as much fluid of the test meal remained in the stomach to dilute the acid, the resulting acidity of the gastric *contents* would be the same as before operation. To avoid misinterpretation a method must be used which will show the acidity of the *secretions* entering the stomach as well as the acidity of the gastric *contents*. 5. Many investigators have confused a lowering of the *amount* of the acid secreted with a lowering of the *concentration* of the acid secretion. Until proven otherwise, it is necessary to assume that any change will involve the amount rather than the concentration of the acid secretion. 6. Most of the results obtained by studies on fundic pouches (transplanted and Heidenhain) are of doubtful significance since they could give positive information only if the stimulating effect of the pylorus operated through a humoral or hormonic mechanism; an unproven assumption.

In the present paper we wish to present studies which appear to avoid the above errors. These studies were planned primarily to answer the question: Is the lowering of acidity which follows partial gastrectomy with Polya anastomosis due entirely to the diluting and neutralizing effect of the increased amount of duodenal secretions entering the stomach?

METHODS. All studies were made with a two percent Liebig's extract test meal containing 15 mgm. of phenol red per liter. The preparation and use of this meal have been described in detail (13) and need not be repeated.

In all animals the meal was introduced and withdrawn by stomach tube or gastrostomy in order to avoid a psychic secretion. The fractional method of analysis was usually employed. Before starting an experiment the stomach was lavaged with 300 cc. of the test meal and from 600 to 900 cc. of meal introduced, depending on the size of the animal. Thirty cubic centimeter samples were withdrawn every half hour until the stomach emptied.

Three groups of studies were made. 1. A comparison of the gastric acidity curve before and after performing gastroduodenostomy with a large stoma in order to allow large amounts of duodenal secretions to enter the stomach. 2. A comparison of the gastric acidity curve before and after partial gastrectomy with a Polya type of anastomosis to the duodenum. 3. A comparison of the acidity curve in whole stomach pouches with and without the pyloric portion.

RESULTS. I. *A Comparison of the Acidity Curve Before and After Gastroduodenostomy and Partial Gastrectomy.* Four dogs were studied, two before and after each type of operation. In table 1 a few typical experiments before and after partial gastrectomy are shown and in table 2 before and after gastroduodenostomy.

TABLE 1

Experiments before and after partial gastrectomy on dog I

P. S. P.	EXTRA ACID CHLORIDE, GASTRIC SAMPLE	ACID CHLORIDE CONCENTRATION OF SECRETION	TOTAL FLUID	ACID FLUID	NON-ACID FLUID	PER CENT ACID FLUID	TIME	BILE	REMARKS
				Before operation					
per cent	*mgm. per 100 cc.*	*mgm. per 100 cc.*							
92	20	250	8	3	5	38	½	0	Average of 7 fractional analyses
80	90	450	20	15	5	75	1	+	
64	167	464	36	28	8	78	1½	+++	
44	210	374	56	35	21	62	2	+++	
78	101	460	22	17	5	77	½	0	"Block" analysis with 300 cc. meal each half hour
79	103	490	21	17	4	81	½	0	
75	103	451	25	19	6	76	½	trace	
78	102	464	22	17	5	77	½	0	
				After partial gastrectomy					
88	−6	−50	12	0	12	0	½	++	Fractional
89	+12	+109	11	2	9	18	1	++	
56	+48	+109	44	8	36	18	1½	+++	
50	+10	+20	50	2	48	4	2	+++	
85	+13	+87	15	2	13	13	½	++	Fractional
71	+56	+193	29	9	20	31	1	++	
48	+100	+192	52	17	35	33	1½	+++	
20	+98	+123	80	16	64	20	2	+++	
98	−4	−200	2	0	2	0	½	trace	Fractional
93	+21	+300	7	4	3	57	1	+	
62	+50	+131	38	8	30	21	1½	+++	
34	+50	+76	66	8	58	12	2	+++	
88	+27	+225	12	5	7	42	½	++	Fractional
66	+52	+153	34	9	25	27	1	+++	
52	+58	+121	48	10	38	21	1½	+++	
48	+14	+27	52	2	50	4	2	+++	
83	+20	+118	17	3	14	18	½	0	Fractional
76	+58	+242	24	10	14	42	1	0	
65	+89	+254	35	15	20	43	1½	0	
32	+98	+144	68	16	52	24	2		
88	+5	+42	12	1	11	8	½	+++	Fractional
66	+26	+77	34	4	30	12	1	++++	
26	+38	+51	74	6	68	8	1½	+++	

TABLE 1—*Concluded*

P. S. P.	EXTRA ACID CHLORIDE, GASTRIC SAMPLE	ACID CHLORIDE CONCENTRATION OF SECRETION	TOTAL FLUID	ACID FLUID	NON-ACID FLUID	PER CENT ACID FLUID	TIME	BILE	REMARKS
After partial gastrectomy—*Concluded*									
per cent	*mgm. per 100 cc.*	*mgm. per 100 cc.*							
91	−3	−33	9	0	9	0	½	+	Fractional
64	+24	+67	36	4	32	11	1	+++	
42	+62	+107	58	10	48	17	1½	+++	
93	+3	+43	7	1	6	14	½	++	Fractional
76	+24	+100	24	4	20	17	1	+++	
58	+12	+29	42	2	40	5	1½	++++	
93	−15	−214	7	0	7	0	½	+++	Fractional
78	+4	+18	22	1	21	5	1	++++	
10	+82	+91	90	14	76	16	1½	++++	
91	+9	+100	9	2	7	22	½	++	Fractional
63	+55	+148	37	9	28	24	1	+++	
34	+26	+40	66	4	62	6	1½	++++	
81	+40	+211	19	7	12	37	½	++	"Block" analysis
64	+48	+133	36	8	28	22	½	+++	with 300 cc. meal
75	+51	+204	25	9	16	36	½	++	each half hour
54	+42	+91	46	7	39	15	½	++++	

After partial gastrectomy there was usually a more rapid emptying of the stomach which shortened the experiment by one-half hour. In dog I (partial gastrectomy), however, a number of experiments were obtained in which the length was the same (2 hours) before and after operation; in other experiments on this dog a shortened experiment was obtained after operation. Dog II (partial gastrectomy) always gave a shorter experiment after operation. In order to produce a similar shortening of the experiment after gastroduodenostomy, in some experiments, less test meal was given so that the emptying time was shortened by one half hour. Thus after both types of operation there are some experiments in which the emptying time is the same as before operation and others in which it is shortened by one half hour.

In column 2 (tables 1 and 2) the extra acid chloride per 100 cc. of gastric contents is shown. When the same animal is compared before and after operation, it is evident that there is a greater lowering after partial gastrec-

TABLE 2

Experiments before and after gastroduodenostomy on dog II

P. S. P.	EXTRA ACID CHLORIDE, GASTRIC SAMPLE	ACID CHLORIDE CONCENTRATION OF SECRETION	TOTAL FLUID	ACID FLUID	NON-ACID FLUID	PER CENT ACID FLUID	TIME	BILE
Before operation								
per cent	*mgm. per 100 cc.*	*mgm. per 100 cc.*						
86	49	350	14	8	6	57	½	0
76	124	516	24	21	3	87	1	0
46	210	389	54	35	19	65	1½	+
89	43	391	11	7	4	64	½	0
75	125	500	25	21	4	84	1	+
52	166	346	48	28	20	58	1½	++
89	48	436	11	8	3	73	½	0
69	128	413	31	21	10	68	1	+
40	190	317	60	32	28	53	1½	++
91	30	334	9	5	4	56	½	+
75	106	424	25	18	7	72	1	++
58	136	324	42	23	19	55	1½	+++
After gastroduodenostomy								
85	37	246	15	6	9	40	½	+++
56	108	246	44	18	26	41	1	+++
20	192	240	80	32	48	40	1½	+++
81	58	306	19	10	9	53	½	++++
56	144	328	44	24	20	55	1	++++
83	43	253	17	7	10	41	½	++
56	104	236	44	17	27	39	1	+++
91	31	345	9	5	4	56	½	++
81	46	242	19	8	11	42	1	+++
66	120	353	34	20	14	59	1½	+++
48	92	177	52	15	37	29	2	+++
69	50	162	31	8	23	26	½	++
52	86	179	48	14	34	29	1	+++
22	182	234	78	30	48	39	1½	++
73	105	389	27	18	9	67	½	++
32	158	232	68	26	42	38	1	+++
76	77	321	24	13	11	54	½	++
61	150	385	39	25	14	64	1	++
40	130	217	60	22	38	37	1½	+++

tomy. However, as mentioned above, this value may be influenced by the amount of fluid from the test meal remaining in the stomach and may thus give an erroneous impression. In column 3 the extra acid chloride per 100 cc. of gastric secretion is shown, this value is independent of the amount of fluid from the test meal remaining in the stomach. This value is lowered after both types of operation but when samples in which the same amount of total fluid entered the stomach are compared it is evident that there is a more pronounced lowering after partial gastrectomy. This is shown graphically in figure 1, where it is seen that the values after partial gastrectomy are usually one-half, or less, than the values after gastroduodenostomy. The lowering after partial gastrectomy is well illustrated in

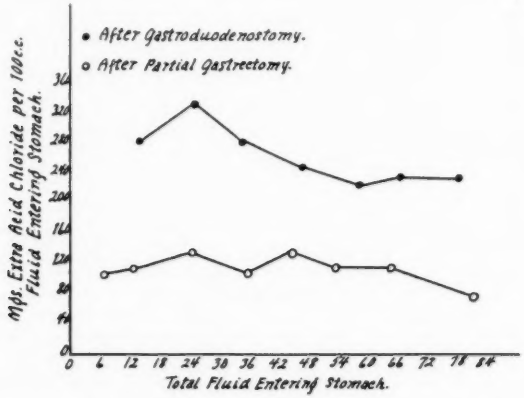

Fig. 1. Shows the acid chloride concentration of the total fluid entering the stomach. The values show group averages for samples containing increasing amounts of total fluid. The time factor was ignored. The analysis is based on 80 half-hour samples from two dogs after partial gastrectomy and 45 half-hour samples from two dogs after gastroduodenostomy.

the "Block" type of experiment. In these 300 cc. of test meal were introduced and allowed to remain for ½ hour, the entire contents were then removed and a second 300 cc. portion introduced and removed after ½ hour. This was repeated every half-hour for two hours.

The above comparisons, based on the acid chloride concentration of similar quantities of total fluid entering the stomach after operation, are open to criticism because the time factor is omitted. In order to include and evaluate the time factor a different type of analysis is necessary. This was done as follows: Average curves for the total fluid, acid fluid and non-acid fluid in each half hour sample before and after operation were made from all experiments on each animal. These average curves were plotted

on coördinate paper as illustrated in figure 2. The areas under the curves are proportional to the amounts of the various secretions and were determined by means of a standardized planimeter and converted into terms of cubic centimeters of the various fluids.

Using the above values it is possible to make several types of calculation to determine definitely whether or not the lowering of the acidity of the gastric secretion is due entirely to the duodenal secretions. The following statement will illustrate the general principle of the calculations: All cal-

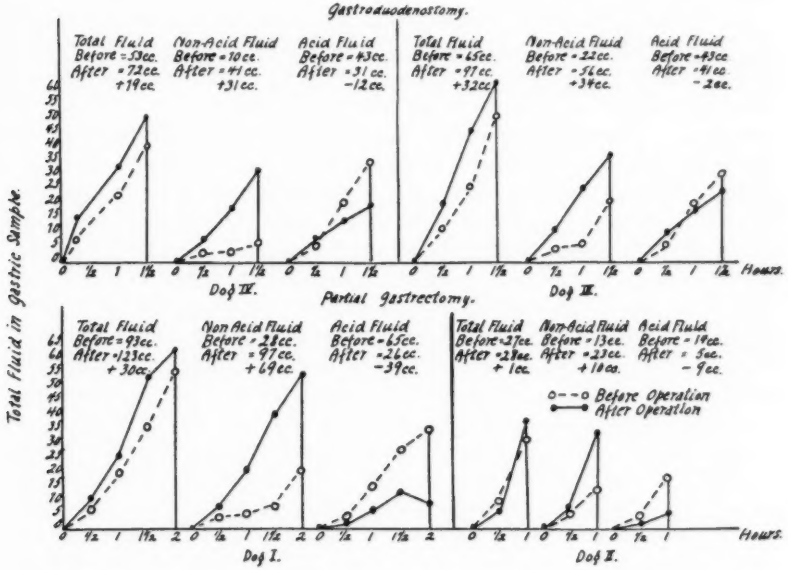

Fig. 2. Average curves for total, acid and non-acid fluid before and after partial gastrectomy and gastroduodenostomy. The areas under the respective curves are proportional to the quantities of the various fluids, these were determined as explained in text.

culations are based on the composition of the total fluid secretions entering the stomach, the acid fluid being expressed as per cent of the total fluid; this is essential in order to eliminate changes due to the different emptying time of the stomach which might influence the acidity of the gastric contents as explained above. After both types of operation there was usually an increase in the total amount of fluid secretions entering the stomach, this increase undoubtedly being due to duodenal secretions. The increased amount of duodenal secretions will lower the per cent of acid fluid in the total fluid entering the stomach both by dilution and neutralization. In

all calculations allowance is made for the diluting effect by determining what the per cent of acid fluid in the total fluid would be if *the amount of acid secreted had remained the same after operation as before*, the acid fluid simply being diluted in a larger amount of total fluid. This is then compared with the per cent of acid fluid actually found after operation. Allowance is made for the neutralizing effect by adding to the acid fluid actually found, the amount which could have been neutralized by the duodenal secretions. Three calculations can be made, the details of which are as follows:

Calculation I. In this it is assumed that the increase in total fluid entering the stomach after operation is due to duodenal secretions. Correction is made for the diluting effect, the neutralizing effect being ignored by assuming that it will be proportionately the same after both types of operation. The method of calculating the diluting effect of the duodenal secretions is illustrated in the following example. Suppose that *before operation* the amount of total fluid entering the stomach was 50 cc. and that the acid fluid was 70 per cent of the total. If *after operation* the amount of total fluid entering the stomach increased to 100 cc., then the acid fluid would be diluted in twice the volume of secretion. *If the amount of acid fluid secreted had remained exactly the same after operation*, then the total fluid should contain 35 per cent of acid fluid (70 ÷ 2). This calculated value is compared with the value actually found. After partial gastrectomy the values found ranged from −32 to −40 per cent while after gastroduodenostomy they ranged from +12 to −20 per cent.

Calculation II. In this it is assumed that the increase in total fluid entering the stomach after operation is due to duodenal secretions and the calculation for dilution is made as illustrated in calculation I. In addition to this a correction is made for the neutralizing effect of the duodenal secretions entering the stomach after operation. Previously reported experiments (14, 15, 16) have shown that the average alkalinity of the duodenal secretions is 0.04 normal. Since the acid, as secreted, is approximately 0.170 normal (17) it follows that 1 cc. of duodenal secretions could neutralize approximately 0.25 cc. of acid fluid. The amount of acid fluid that could have been neutralized by the increased total fluid entering the stomach is calculated and added to the amount actually found. This value is then expressed as per cent of acid fluid in the total fluid and compared with the percentage (corrected for dilution) that should be found had the acid secretion remained unchanged after operation. After partial gastrectomy the values ranged from −25 to −38 per cent while after gastroduodenostomy they ranged from +15 to −16 per cent.

Calculation III. In this the diluting effect of the increase in total fluid after operation is allowed for as in calculations I and II. In addition, however, a correction is made for the neutralizing effect of the non-acid

fluid in the secretion both *before* and *after* operation. The non-acid fluid is assumed to have an alkalinity of 0.04 normal and since (as explained above) 1 cc. of this will neutralize approximately 0.25 cc. of acid fluid, which then becomes non-acid fluid, it follows that each 1.25 cc. of non-acid fluid represents 0.25 cc. of neutralized acid fluid or that 1 cc. of non-acid fluid represents 0.20 cc. of neutralized acid fluid. Therefore for every cubic centimeter of non-acid fluid in the samples 0.2 cc. of acid fluid is added to the observed acid fluid. The corrected acid fluid is then expressed as per cent of the total fluid and compared with the per cent that should have been found (corrected for dilution) had the acid secretion remained the same after operation as before. The values after partial gastrectomy ranged from −20 to −32 per cent while after gastroduodenostomy they ranged from +20 to −12 per cent.

Each of the above calculations is theoretically justified, II and III being perhaps the most accurate. The value used for the alkalinity of the non-acid fluids is the average value obtained in 95 experiments previously reported (18). The alkalinity of the non-acid fluids entering the stomach was actually determined (using an acid Liebig's extract test meal) on the two animals before partial gastrectomy. Seven determinations on dog I gave an average value of 0.05 normal while 9 determinations on dog II gave an average of 0.03 normal. From this it is clear that the corrections for the neutralizing effect of the non-acid fluids are quite accurate.

The above experiments appear to justify the conclusion that after removal of the pyloric segment, the acidity of the gastric secretion is reduced more than can be accounted for by the diluting and neutralizing effect of the increased duodenal secretions entering the stomach.

II. *The Acidity Curve in Whole Stomach Pouches with and without the Pyloric portion.* The indirect method of analysis used in the above experiments required verification by more direct methods, hence the experiments on whole stomach pouches were performed.

Nine pouches were studied. In six the stomach was separated from the duodenum but remained attached to the esophagus and opened to the surface by a gastrostomy. The nerve supply, both vagus and sympathetic, was intact. In three of these the pyloric portion was attached while in three others it was removed (lower half fig. 3). In three other pouches the stomach was separated from the duodenum and opened to the outside by a gastrostomy. The esophagus and a small portion of the cardiac end of the stomach were anastomized to the duodenum. These pouches were partially (vagus) denervated. In one the pylorus was attached while in two others it had been removed (upper half fig. 3).

The pouches were thoroughly lavaged with a portion of the test meal before starting an experiment. From 100 cc. to 300 cc. of test meal were introduced. Samples were removed every half hour for two hours, the

amount removed each half hour was the same, being such as would empty the pouch with removal of the fourth sample. All animals were in good condition when the experiments were performed.

The results are self evident and need no explanation. In unpublished experiments on the same type of preparation, Ivy (21) has obtained similar results. The cause of the residual secretion after pylorectomy is not clear. It is not due to distention of the pouch since reduction of the

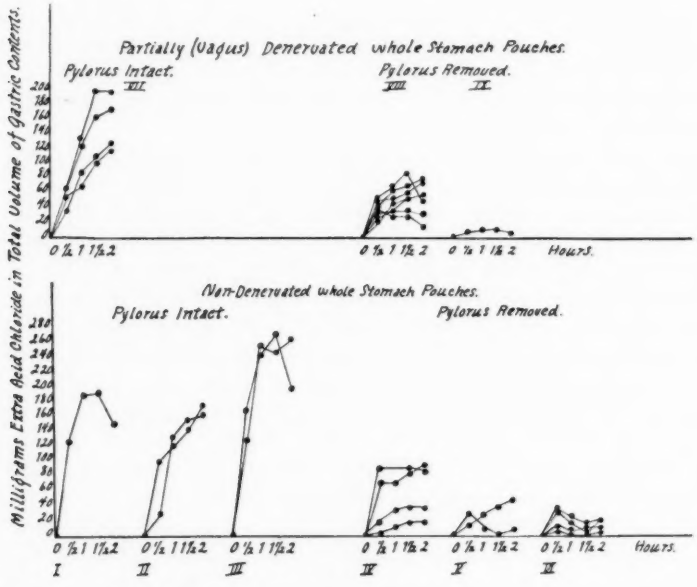

Fig. 3. *Lower half.* The total amount of acid chloride secreted by non-denervated whole stomach pouches with and without the pylorus.

Upper half. The total amount of acid chloride secreted by partially (vagus) denervated whole stomach pouches with and without the pylorus.

amount of meal introduced from 250 or 300 cc. to 100 cc. caused no reduction in the amount of secretion.

In figure 3 the results are expressed as the total amount of extra acid chloride in the total volume of fluid in the pouch. It was necessary to use this method of expression in order to make all experiments strictly comparable because absorption of water from the pouch contents (13) interfered with the expression as milligrams of extra acid chloride per 100 cc. of secretion, while the different volumes of test meal introduced (100 cc. to 300 cc.) in different experiments made it impossible to express the results as milligrams of extra acid chloride per 100 cc. of gastric contents and have comparable figures.

DISCUSSION. The experiments reported show quite clearly that the pyloric segment exerts a specific stimulating influence on the acid secreting mechanism of the fundus and is thus of importance in the intragastric chemical phase of acid secretion. The mechanism of this effect is unknown. Many investigators have assumed that the mechanism depends upon humoral or hormonic effects and have planned their experiments on this assumption. There is, however, evidence in the work of Kim and Ivy (19) and of Wilhelmj, O'Brien and Hill (20) which suggests that secretogogues bring about the intragastric chemical phase of acid secretion by nervous mechanisms involving the intrinsic nerve plexus of the stomach. Thus since the pylorus is involved, its rôle may be a reflex one.

Priestley and Mann (12) who found no change in the acidity of the gastric contents after partial gastrectomy give four reasons for the lowered acidity found by others. Three of these are of interest in connection with the present experiments: 1. *That some fundic mucosa is frequently removed in the operation.* In the present experiments special care was taken to avoid this. 2. *That dilution and neutralization by duodenal fluids are responsible.* The present experiments eliminate this as the sole factor. 3. *That after partial gastrectomy food leaves the stomach sooner and therefore stimulates gastric secretion for a shorter period of time.* This factor was eliminated by the experiments after gastroduodenostomy in which we introduced less meal and shortened the experiment to the same extent as occurred after partial gastrectomy but failed to reduce the acidity of the secretions entering the stomach to the same extent as occurred after partial gastrectomy.

The duration of the lowered acidity after partial gastrectomy was not specifically studied but it was found to persist for at least 8 months.

After partial gastrectomy there occurred a marked change in the consistency of the gastric contents. They become very mucoid and thick due to the presence of large amounts of mucus having the consistency of raw egg white. This change was noted shortly after operation in dog II and developed gradually in dog I. In both dogs it persisted for at least 8 months.

The cause of the residual acid secretion after partial gastrectomy is not clear. In six experiments on both dogs it was completely abolished by atropine (0.05 mgm. per kilo) which suggests that it may have been cephalic (not psychic) in origin, although Ivy (21) finds that atropine may abolish all phases of secretion in the dog.

SUMMARY

1. The effect of the pylorus on the acid secreting mechanism of the fundus has been investigated using new methods.

2. A comparison of the acid secretion in two dogs before and after partial gastrectomy with that in two dogs before and after gastroduodenostomy showed that after removal of the pylorus the acid secretion was lowered

more than could be accounted for by the diluting and neutralizing effects of the duodenal secretions entering the stomach.

3. A study of four whole stomach pouches with the pylorus intact and five with the pylorus removed showed that the acid secretion was markedly lowered by removal of the pylorus.

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Editorial Policy. The Council has approved the following policy of management:

Manuscripts should be sent to the Managing Editor who will see that each paper is read by two or more members of the Editorial Board. Authors will then be advised as to the suitability of the paper or the desirability of any revision. The Editorial Board will be governed by certain general principles:

1. The suitability of papers will not be judged by arbitrary standards of length but on their content of significant new research results in physiology, presented with the greatest brevity which is compatible with scientific accuracy and clarity.

2. Preference will be given to papers from American Laboratories in the field of vertebrate physiology and to those which contribute to problems related to this field.

3. Subdivision of material coming out of a general research into two or more papers will be discouraged.

4. Papers restricted to the description of new apparatus or methods or which appear to be of the nature of progress reports, the publication of which might properly be withheld until the research has progressed to the completion of at least a significant phase of the problem, will not be accepted.

5. Papers giving confirmatory or negative results will be considered only if presented in the briefest possible space.

6. Since manuscripts will not be insured against loss or injury when being given editorial consideration, contributors will be expected to retain duplicate copies, either originals or photographs, of all material (manuscripts, illustrative and tabular matter) submitted for publication.

The following practical aspects are important in the preparation of papers:

a. Duplication of data in tables, charts and protocols is not believed to be generally necessary. Too extensive use of such material is likewise to be deprecated.

b. Tables and illustrative material should be prepared with the size of the Journal page ($4\frac{1}{2}$ x $7\frac{1}{2}$ inches) in mind, specifically with the idea of conserving vertical space.

c. It is advantageous, when feasible, to group illustrations. This should be done with as little waste space between the several units as is possible and also with the idea of conserving vertical space.

d. Since duplication of charts, graphic tracings, etc., is required in paragraph six above, this is best done by photographs of the size desired in reproduction and printed on glossy paper. Either the originals or photographs may be submitted. If the originals are larger than $8\frac{1}{2}$ x 11 inches they must in all cases be accompanied by photographic reproductions. When such photographs are adequate for good reproduction the originals need not be supplied.

e. Plotted curves and their guide-lines should be drawn in India ink on blue-lined coördinate paper.

f. All illustrative material must be submitted in such form as to admit of photographic reproduction without retouching, redrawing or the setting of marginal type.

g. References to cited papers should conform to the practice of the Quarterly Cumulative Index Medicus and should be in this order: Author's name, journal, volume (in Arabic), initial page, year.

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